Exploring the relevance and extent of small airways dysfunction in asthma (ATLANTIS)

ATLANTIS study group; Postma, Dirkje S; Brightling, Chris; Baldi, Simonetta; Van den Berge, Maarten; Fabbri, Leonardo M; Gagnatelli, Alessandra; Papi, Alberto; Van der Molen, Thys; Rabe, Klaus F

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Corresponding Author: Dr. Dirkje Postma, Ph.D.

Corresponding Author's Institution: University of Groningen, University Medical Center Groningen

First Author: Dirkje Postma, Ph.D.

Order of Authors: Dirkje Postma, Ph.D.; Chris Brightling; Simonetta Baldi; Maarten van den Berge; Leonardo Fabbri; Alessandra Gagnatelli; Alberto Papi; Thys van der Molen; Klaus Rabe; Salman Siddiqui; Dave Singh; Gabriele Nicolini; Monica Kraft

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Abstract: Background
Small airways dysfunction (SAD) is well-recognized in asthma, yet its role in severity and control is unclear.
Methods
This multinational observational study investigated participants without and with asthma (GINA 1-5). They underwent spirometry, body plethysmography, impulse oscillometry (IOS), Multiple Breath Nitrogen Washout (MBNW), computed tomography (CT) and questionnaires. Structural equation modeling in asthma, applied to the physiological and CT parameters, defined a clinical-SAD and CT-SAD score. Asthma subjects were classified in SAD groups using model-based clustering. Asthma severity, control and health care utilization in the past year were compared with the SAD scores and SAD groups.
Findings
We investigated 773 asthma and 99 control participants (median [interquartiles] age 46 [34, 54] and 41 [29, 52] years, 58% and 57% females, respectively). All physiologic measures contributed to the clinical SAD model; SAD prevalence was dependent on the measure used. IOS and spirometry contributed most to the Clinical-SAD score and SAD Groups. Clinical-SAD Group1 (n=452) had comparable MBNW Sacin values with controls. Group2 (n=312) had more abnormal physiologic SAD measures than Group1, particularly IOS and spirometry, and more severe asthma (asthma control, treatments, exacerbations, quality of life). Clinical-SAD scores were higher in Group2 and related to asthma control, severity, and exacerbations. Clinical-SAD and CT-SAD scores did not significantly correlate.
Interpretation

SAD has multiple components, is present across all asthma severity and particularly in severe disease. The clinical classification of SAD, by the easy-to-conduct measures IOS and spirometry, is meaningful given its association with asthma severity, control, quality of life, and exacerbations.

Exploring the relevance and extent of small airways dysfunction in asthma: baseline data from the AssessmenT of smalL Airways involvemeNT In aSthma (ATLANTIS) prospective cohort study

Postma DS[1], MD Prof, Brightling C[2], MD Prof, Baldi S[2,11], MD PhD, Van den Berge M[1] , MD, Fabbri LM[3,4], MD Prof, Gagnatelli A[5], PhD, Papi A[6], MD Prof, Van der Molen T[7], MD, Prof, Rabe KF[8], MD Prof, Siddiqui S[2],MD, Singh D[9], MD Prof, Nicolini G[5], PhD, Kraft M[10] , MD Prof, and the ATLANTIS study group*

Author Information

1. University of Groningen, University Medical Center Groningen, Department of Pulmonology, Groningen Research Institute for Asthma and COPD, Groningen, The Netherlands University of Groningen, University Medical Center Groningen, Department of Pulmonology, Groningen Research institute for Asthma and COPD, Groningen, The Netherlands
2. Institute for Lung Health, NIHR Biomedical Research Centre, University of Leicester, UK; Department of Global Clinical Development, Chiesi SAS, Bois Colombes, France
3. Research Centre on Asthma and COPD, University of Ferrara, Ferrara, Italy
4. COPD Centre, Sahlgrenska University Hospital, Gothenburg
5. Department of Global Clinical Development, Chiesi Farmaceutici SpA, Parma, Italy
6. Research Centre on Asthma and COPD, University of Ferrara, Ferrara, Italy
7. University of Groningen, University Medical Center Groningen, Department of General Practice, Groningen Research Institute for Asthma and COPD, Groningen, The Netherlands
8. Lungen Clinic, Grosshansdorf, and Department of Medicine, Christian Albrechts University, Kiel, Germany. Airway Research Center North in the German Center for Lung Research (DZL), Germany.

9. Centre for Respiratory Medicine and Allergy, University of Manchester, University Hospital of South Manchester, Manchester, UK.
10. University of Arizona College of Medicine, Department of Medicine, Tucson, Arizona, United States
11. Department of Global Clinical Development, Chiesi SAS, Bois Colombes, France

Link to full study:

https://clinicaltrials.gov/ct2/show/NCT02123667?term=NCT02123667&rank=1

Summary

Background

Small airways dysfunction (SAD) is well-recognized in asthma, yet its role in asthma severity and asthma control is unclear. Our study aimed to assess which (combination of) biomarkers, physiological testing and imaging markers best measures the presence and extent of SAD in asthma.

Methods

This multinational observational study investigated participants without and with asthma (GINA severity stage 1-5). Asthma inclusion criteria were: 1) age 18-65 years; 2) clinical asthma diagnosis > 6 months, confirmed by a chest physician 2, supported by objective evidence of any of the following at the baseline visit or in the previous 5 years: a) positive airway hyperresponsiveness to methacholine, ***or*** b) positive reversibility ($\Delta FEV_1 \geq$ 12% and ≥ 200 mL within 30 minutes after 400 µg of salbutamol pMDI with or without a spacer ***or c)*** PEF variability >20%, measured during 7 days ***or*** d) documented reversibility after a cycle (e.g. 4 weeks) of maintenance anti-asthma treatment; 3) stable asthma on any previous regular asthma treatment ("rescue" β2-agonists alone included) at a stable dose for > 8 weeks before baseline; 4) lifetime smoking ≤ 10 pack-years. They underwent spirometry, body plethysmography, impulse oscillometry (IOS), Multiple Breath Nitrogen Washout (MBNW), computed tomography (CT) and questionnaires. Structural equation modeling (SEM) was applied in asthma to assess the contribution of all physiological and CT parameters to SAD. With SEM, we defined a clinical-SAD and CT-SAD score. Asthma subjects were classified in SAD groups using model-based clustering. Asthma severity, control and health care utilization in the past year were compared with the SAD scores and SAD groups.

Findings

We investigated 773 asthma and 99 control participants (median [interquartiles] age 46 [34, 54] and 41 [29, 52] years, 58% and 57% females, respectively). All physiologic measures contributed to the

clinical SAD model with SEM analysis. The prevalence of SAD in asthma was dependent on the measure used and lowest with MBNW Sacin that reflects ventilation heterogeneity in the most peripheral, pre-acinar/acinar airways. IOS and spirometry, reflecting dysfunction of small-to-mid-sized airways, contributed most to the Clinical-SAD score and differentiated the two SAD Groups. Clinical-SAD Group1 (n=452) had "milder" SAD, i.e. comparable MBNW Sacin with controls. Group2 (n=312) had more abnormal physiologic SAD measures than Group1, particularly IOS and spirometry, and more severe asthma (asthma control, treatments, exacerbations, quality of life). Clinical-SAD scores were higher in Group2 ("more severe" SAD) and related to asthma control, severity, and exacerbations. Clinical-SAD and CT-SAD scores did not significantly correlate.

Interpretation

SAD is a complex and silent signature of asthma, which is likely to be directly or indirectly captured by combinations of physiologic tests: spirometry, body plethysmography, IOS, and MBNW. SAD is present across all asthma severity and particularly in severe disease. The clinical classification of SAD in two groups, i.e. a "milder" and "more severe" SAD group, by the easy-to-conduct measures IOS and spirometry, is meaningful given its association with GINA asthma severity stages, asthma control, quality of life, and exacerbations. The longitudinal part of ATLANTIS will show the relevance of the SAD score for future risks in asthma, and additionally which parameter best associates with future asthma control. Moreover, we will report on development of a Small Airways Dysfunction Tool (SADT), a questionnaire as an easy measure to suggest SAD, and on the measures of inflammation that best discriminate between the large and small airways' compartments, with bronchial and transbronchial biopsies, in a smaller subset of participants.

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Research in context

Evidence before this study

We searched PubMed for studies in asthma, including the terms asthma, adult, and small airways, and published between database inception and April 2018, using spirometry and any combination of body plethysmography, impulse oscillometry (IOS; including R5-R20 values) and Multiple Breath Nitrogen Washout (MBNW) measures, and similar terms in addition to CT scans. Small airways dysfunction (SAD) has been understudied, though it significantly contributes to airway obstruction, a hallmark of asthma. So far, studies on the role of SAD in asthma have been performed in small sample sizes and/or subgroups of asthma. Moreover, these studies investigated only a subset of available potential measurements of SAD and did not include both spirometry, body plethysmography, IOS, MBNW, CT scans and questionnaires.

Added value of this study

This is the largest study to date involving 773 evaluable asthma patients and 99 controls without airway obstruction specifically designed to determine the prevalence and impact of small airways dysfunction SAD in asthma. The study shows that SAD is present in asthma across all stages of severity, with highest prevalence in GINA 5. We were able to define a SAD score from a combination of lung function measurements that reflects the amount of physiological small airways impairment in asthma. The score associated significantly with measures of asthma control, history of exacerbations and disease severity. Model-based clustering delineated two clinical SAD groups that differed in age, duration of asthma, and disease severity. Of interest, values of Sacin, that measures ventilation heterogeneity in pre-acinar/acinar airways, were in the normal range in Group1. The difference between Clinical SAD Group1 and Group2 was particularly clear with clinically available SAD measurements, such as IOS and spirometry, followed by FEV_1, while

differences were small with CT SAD parameters. In summary, we can cluster asthma patients in two subgroups based on SAD measured with easy-to-conduct, clinically applicable measures.

Implications of all the available evidence

Small airways dysfunction (SAD) has been understudied in asthma. Our results show the clinical relevance of SAD, which is present across all severity stages of asthma. It is particularly present in severe disease, likely reflecting structural lung changes that are not responsive to the use of oral corticosteroids and/or high dose inhaled corticosteroids. Moreover, SAD relates to asthma stability, severity, quality of life, exacerbation rates and health care utilization and can be delineated by easy-to-conduct, clinically applicable measures such as IOS and spirometry. Therefore, this aspect of asthma needs further consideration in the management of the disease.

Introduction

Asthma is a prevalent obstructive airway disease that affects the entire bronchial tree. The small airways, defined by a diameter ≤2 mm and referred to as the "silent zone" of the lungs, contribute to the resistance in the airways of patients with obstructive airways disease[1]. This is of clinical importance since small airways can be inflamed in asthma and hence narrowed[2-4]. Small airway narrowing can also occur due to smooth muscle contraction after inhaling allergic and non-allergic irritants. Moreover, remodeling can affect small airway wall stiffness, thereby changing their distensibility[5].

Small airways dysfunction (SAD) has been postulated to exist at all severities of asthma, whereas some studies suggest that the prevalence increases with asthma severity [6,1]. However, it is not clear what proportion of asthma patients suffers from SAD, and which tests or combination of tests best defines it. Lack of best practice is due to the fact that published studies investigating the small airways in asthma included only small-sized and/or relatively homogeneous populations regarding asthma severity, or only tested one or a few physiologic SAD measures [6-8]. The ATLANTIS (AssessmenT of smalL Airways involvemeNT In aSthma) study is a multinational 1-year prospective cohort study, including people with asthma of all severities and controls without airway disease. In this paper we present the baseline, cross-sectional data from ATLANTIS, aiming to identify which combination of biomarkers, physiologic testing and imaging approaches best measures the presence and extent of SAD in asthma, and their relationship with features of asthma. We assess SAD through a series of all available, clinically applicable, potential SAD tests, both for physiological and CT measures. The physiological tests may reflect abnormalities in different parts of the bronchial tree or different aspects of small airways dysfunction, providing different perspectives on SAD[9,10]. Lung imaging by CT scan can provide additional insight regarding SAD, but the relationship with physiologic measures of SAD in asthma has not been studied extensively and only in small groups; here we test both physiological and CT scan measures in a large cohort.
In addition, we develop a score defining to what extent SAD is present in each individual patient and assess

its usefulness for prediction of asthma severity, control, quality of life and history of exacerbations. In future papers (not presented here), we will report the longitudinal data from ATLANTIS and aim to validate the SAD score over time, we will develop and validate a Small Airways Dysfunction Tool (SADT), a questionnaire as an easy measure to suggest SAD, and we will assess which direct and indirect measures of inflammation best discriminate between the large and small airways' compartments, with bronchial and transbronchial biopsies, in a smaller subset of participants[9].

Methods

Participants

Participants were recruited (first patient in June 30, 2014 and last patient out March 3, 2017) from general practitioners, chest physician's databases and by advertisements in 29 centers across 9 countries worldwide. Inclusion criteria were: 1) age 18-65 years; 2) clinical asthma diagnosis ≥6 months, confirmed by a chest physician according to GINA 2012[11] and supported by objective evidence of any of the following at the baseline visit or in the previous 5 years: a) positive airway hyperresponsiveness to methacholine, ***or*** b) positive reversibility, defined as $\Delta FEV_1 \geq$ 12% and ≥ 200 mL over baseline FEV_1 within 30 minutes after inhaling 400 μg of salbutamol pMDI with or without a spacer ***or c)*** Peak Expiratory Flow variability (i.e. highest - lowest value over the day/mean value of the two, ×100) >20%, measured during 7 days ***or*** d) documented reversibility after a cycle (e.g. 4 weeks) of maintenance anti-asthma treatment; 3) stable asthma on any previous regular asthma treatment ("rescue" β2-agonists alone included) at a stable dose for ≥ 8 weeks before baseline; 4) lifetime smoking ≤ 10 pack-years. Main exclusion criteria were a COPD diagnosis confirmed by a chest physician and an asthma exacerbation during 8 weeks before baseline.

Controls were included based on 1) age 18-65 years; 2) no respiratory symptoms compatible with asthma or COPD in the past 2 years; 3) normal spirometry: baseline $FEV_1 \geq$ 80%predicted, FEV_1/Forced Vital Capacity (FVC)> LLN (lower limit of normal); 4) normal airways responsiveness: PC20≥ 16 mg/mL, PD20≥ 1.4 mg; 5) lifetime smoking ≤ 10 pack-years. Diagnosed upper/lower respiratory tract diseases were exclusion criteria. The Medical Ethics Committee of each center approved the protocol; all patients gave written informed consent.

Study design and procedures

Participants were followed for 1 year with 6-month clinic and 3-month telephone follow-ups[9]. The clinical and CT tests were performed at 3-day baseline visits. The methods for spirometry,

hyperresponsiveness, MBNW, IOS, body plethysmography, CT, questionnaires, blood tests, and health care utilization are described in the Supplement. Medications during an eight-week period before evaluation were used to assess GINA severity[11]. The potential indices of SAD used with hypothetical location in the airways and references between brackets are presented in Table 1 [12-19]. These were % fall in FVC during hyperresponsiveness testing; spirometry: Forced Expiratory Flow $(FEF)_{25-75,}$ FEF_{50}, both corrected for FVC; body plethysmography: Residual Volume/Total Lung Capacity (RV/TLC), Functional Residual Capacity (FRC), IOS: R5-R20, AX, X5; MBNW: Scond, Sacin. Alveolar NO was not incorporated in this analysis since it was only available in a subset of participants (Supplement). Indices of "large airways dysfunction", which may also capture small airways abnormalities, were FEV_1%predicted, FEV_1/FVC, IVC, FeNO, R20, PC_{20}, PD_{20} and 3 severity categories of airway hyperresponsiveness (Supplement).

Computed tomography

Volumetric whole lung scans were obtained at full inspiration (near total lung capacity) and at end of expiration, near FRC. Scans were analyzed by a single observer (SB) using semi-automated software, Apollo (VIDA Diagnostics, Iowa), with various quality control parameters[20,21]. The supplement describes CT acquisition, quantitative airway morphometry and lung densitometry. SAD parameters used (Table 1 [21,22]) were: ex- and inspiratory Mean Lung Density and their ratio (E/I MLD), ex- and inspiratory lung volume and their ratio (E/I LV), expiratory Voxel Index (VI-856) and inspiratory VI-950 (% of Voxels with CT numbers <-856 and <-950 Hounsfield Units respectively, inspiratory Percentile15, Inspiratory median Lumen area, Wall area (WA) and Total area, these latter three divided by body surface area (BSA), inspiratory median percentage WA, and inspiratory Pi10 and Po20%WA (hypothetical airway with internal perimeter of 10 mm and outer perimeter of 20 mm respectively).

Statistical analyses

Detailed statistical information, including power analysis[23], is provided in the Supplement. The following variables reflecting SAD were used in the clinical SAD analysis: FEF_{50}/FVC, FEF_{25-75}/FVC, FEV_1%predicted, FEV_1/FVC, IVC%predicted, % fall FVC at PC_{20} or PD_{20}, RV/TLC %predicted, FRC%predicted, R5-R20, X5, AX, Scond, Sacin. For CT SAD analysis, variables were: MLD ratio, Lung Volume ratio, VI-856, Pi10, Po20%WA.

We used SEM analysis to assess clinical SAD, since this clarifies which SAD parameters, out of all the physiologic parameters measured, group together and weigh towards the presence of SAD in asthma (Supplement). Similarly we applied SEM analysis for CT SAD. Several steps were performed for clinical SAD and CT SAD SEM analysis separately[24]. A correlation matrix evaluated correlations among observed variables, high correlations indicating presence of underlying latent variables. An exploratory factor analysis for observed variables was performed to identify the underlying SAD factor structure. The final underlying SAD factor structure was tested by specifying a confirmatory factor model. Once the measurement model was set and fit the data properly, it was used to classify each patient into SAD groups, using model-based clustering. The SAD Groups and SAD scores from the clinical SAD and the CT-scan SAD model were compared, evaluating the rate of agreement, using Chi-square and Pearson's correlation tests. The clinical SAD model was additionally tested in the subgroup with a CT scan, by adding the CT scan variables to the model. Full information maximum likelihood (FIML) method was used for dealing with missing data in SEM analysis[25].

Relationships of physiologic SAD variables with asthma severity, control and healthcare utilization were analyzed by Poisson regression. Continuous prediction equations, their lower- and upper limit of normal (LLN and ULN) from the literature[26] and from formulas based on ATLANTIS controls are provided in Supplemental Table 1. Statistical analyses and data processing were performed using Statistical Analysis Systems (SAS®) Software (release 9.2) and Mplus Version 7.4 on a Windows 7 operating system.

Role of Funding Source

Chiesi Farmaceutici SpA financed the study, contributed to the set-up of the study which was designed by DP, MK, CB, MvdB, LF, AP, TvdM, KR, SS and DS. Chiesi Farmaceutici SpA contributed to interpretation of the study and approved the submitted manuscript. Data collection and management was done by Cromsource and data were analysed by CROS NT. All co-authors discussed and interpreted the data. The first draft of the report was written by DP, CB and MK; DP collated input from all co-authors. DP and MK had access to raw data. The corresponding author had full access to all of the data and the final responsibility to submit the initial and revised manuscript.

Results

The main reason for screening failure was not fulfilling inclusion/exclusion criteria (n=99, Figure 1).

Participants

Baseline characteristics are shown in Table 2, Table 3 (asthma only) and Supplemental Table 2. Gender, age and smoking habits were comparable between asthma and control participants; the large majority of people included were of Caucasian descent (88% and 96% in asthma and control participants respectively). Asthma participants demonstrated higher BMI, heart rate, blood pressure, blood cell counts, and prevalence of atopy. Hyperresponsiveness was only present in asthma participants. All physiologic parameters were significantly worse in asthma. Asthma participants had lower MLD expiratory values, inspiratory airway lumen, wall, and total area, also when divided by BSA on CT. Asthma participants had a moderately severe health status impairment (Table 3) and lower lung-related quality of life (higher EuroQol-5Dim-5Levels score) than controls, median (Q1;Q3) being 95.0 (90.0;100.0) versus 80.0 (70.0;90.0).

Association of physiologic parameters with asthma severity, control and health care utilization

X5, Scond, RV/TLC, R5-R20 and R5 values (Figure 2A) showed the highest positive correlations with GINA severity[11]. GINA severity was also associated, as expected, with lower FEV_1, FEF_{50}, and FEV_1/FVC values. Table 4 shows that GINA5 had the highest SAD prevalence rate for every physiologic variable (measurements >ULN or <LLN). Sacin had the least SAD prevalence rate in all GINA stages, the lowest prevalence being with GINA1 (12%), rather similar, higher prevalences in GINA2-4 (18-19-20%), and highest in GINA5 (41%). This contrasts with other SAD variables, where prevalences either remain constant over the GINA stages (% fall FVC), continuously increase from GINA1-GINA5 (body plethysmography), or increase in steps, e.g. Scond and FEF_{25-}

$_{75}$ showed lowest prevalences in GINA1-2, higher in GINA3-4 and highest in GINA5. R5-R20 and AX showed somewhat comparable rates in GINA1-3, higher in GINA4 and highest in GINA5 (Table 4). Sacin also contrasted with <LLN prevalence distributions in FEV_1, i.e. GINA1-GINA5 26%-29%-36%-47%-72%.

A lower Asthma Control Test (ACT) score was particularly associated with higher AX and R5 and lower FVC and FEV_1 (Figure 2B).

For exacerbations in the past year, highest positive correlations were with RV/TLC, R5-R20, AX and Sacin and highest negative correlations with FEV_1, FVC, IVC, FEF_{25-75}, FEF_{50} (Figure 2C). The number of exacerbations was independently predicted by SAD parameters from spirometry, IOS, body plethysmography, hyperresponsiveness severity, female gender and height (Table 5). There was also a negative association with Raw. Independent parameters for unscheduled consultation visits were FEV_1, hyperinflation with body plethysmography, hyperresponsiveness severity, and female gender (Table 5).

Prevalence of LAD and SAD in asthma

Figure 3 (upper panel) shows the prevalence rates of large and small airways dysfunction, based on LLN and ULN. Sacin had the lowest SAD prevalence (19.2%), % fall FVC the highest (73.1%).

SAD Model

Figure 4 shows the final clinical SAD model based on cross-sectional data. It presents both the loadings to the three latent variables and the goodness of fit values (Supplemental methods), showing good coherence of this model to SAD. IOS parameters R5-R20, AX and X5 loaded to the first latent variable, FEF_{50} and FEF_{25-75} both corrected for FVC, to the second latent variable, while Sacin (MBNW) loaded both to the first and second latent variable. The lung volume parameter RV/TLC %predicted and Scond (MBNW) loaded to the third latent variable. Hyperresponsiveness was only tested at the first visit, hence could not be taken into account in the longitudinal design of

the SAD SEM model. Therefore, we also analyzed the clinical SAD model at baseline including hyperresponsiveness, and the % fall FVC loaded on the third latent variable without much change in goodness of fit values. The baseline model without and with % fall FVC correlated highly (r=0.99; Supplemental Figure 2A). Since the cross-sectional SAD model with and without % fall FVC were almost identical, the model without % fall FVC was tested longitudinally; the same model structure was confirmed at all visits (Supplemental Figure 2B).

Correlations of clinical SAD score with physiologic and clinical parameters

A higher clinical SAD score reflects more severe SAD. The highest positive and negative correlations ($r > 0.60$ and $r < -0.60$) of the SAD score existed with physiologic parameters on which the score was based, i.e. IOS parameters AX, R5-R20, and R5 (positively) and X5, spirometric parameters FEF_{25-75} and FEF_{50} (negatively), next being FEV_1 %predicted (Figure 5). The highest correlations of non-physiological parameters with the SAD score were duration of asthma, ACQ-6 and number of exacerbations (positively), ACT, Mini AQLQ total and EQ-5D-5L (negatively). Clinical SAD scores increased with higher asthma severity, mean SAD score in GINA1-5 being -0.143, -0.035, -0.048, +0.071 and +0.239 (ANOVA $p < 0.0001$).

Model-based clustering defined clinical SAD Groups

Model-based clustering defined two clinical SAD groups, Group1 including 452 patients, Group2 312 patients (Table 6 and Supplemental Table 3 present clinical characteristics). Overall, the 2 clinical SAD Groups were similar regarding age of asthma onset, sex ratio, FeNO, atopy, and smoking habits, while duration of smoking was higher in Group2 (Table 6). Sacin values were comparable between Group1 and the controls, whereas Group2 had significantly higher values than both Group1 and controls. Clinical SAD Group2 was somewhat older, demonstrated higher blood pressure, heart rate and BMI, and a longer asthma duration. Additionally, Group2 had more severe asthma than Group1, according to GINA severity, ACT, ACQ, LABA/ICS use,

hyperresponsiveness, blood inflammation (eosinophils), quality of life and health care utilization. All physiologic parameters were worse in clinical SAD Group2; the two groups were best separated by SAD parameters from IOS followed by spirometry, and additionally FEV_1 (Figure 3).

CT scan factors in SAD

CT scans were analyzed in 294 patients (with comparable asthma severity as the non-CT group, Supplemental Table 3). The SEM model provided three factors in CT that contributed to SAD: MLD inspiratory/expiratory ratio, Lung volume inspiratory/expiratory ratio and VI-856 (Supplemental Figure 2D). The correlations of the CT SAD score with physiologic and clinical parameters, comparison of CT SAD groups, and additional Clinical SAD analysis in patients who had a CT scan are presented in the Supplement.

Relationship between Clinical and CT SAD scores

The Clinical SAD and CT SAD scores showed a significant, weak correlation (r=0.28). There was no significant overlap between the clinical SAD and CT SAD Groups (p=0.10, Supplemental Table 5).

Discussion

This large study shows the clinical relevance of small airway dysfunction for asthma, since SAD is present across all severities and particularly in more severe asthma. ATLANTIS was specifically designed to determine the prevalence and impact of SAD in asthma and has performed the most comprehensive evaluation of SAD to date using both physiological and imaging tools. We show that the prevalence of SAD depends on the physiologic measure used, i.e. localization and type of airway narrowing. Of importance, no single variable defines SAD, but IOS, MBNW, lung volumes and spirometry all contribute. For clinical practice, it is important to highlight that SAD associates with GINA severity and -independently- with history of exacerbations over time, particularly when measured by IOS, spirometry and body plethysmography. Moreover, the poorest asthma control was present in the group with the worst clinical SAD score.

Of note, 91% of our asthma population expressed SAD, ~~when~~ defined as any abnormal physiologic parameter. This does not imply that patients ~~Our data imply that they do~~ have extensive SAD throughout all airway dimensions, since the prevalence varied with the type of physiologic measure. The lowest prevalence existed with Sacin (19%) and RV/TLC (22%), both reflecting dysfunction of the most peripheral small airways. The highest prevalence was with FEF_{25-75} (68%) and % fall FVC (73%), probably both reflecting obstruction in more small-to-mid-sized airways. Future work has to elucidate if these different prevalence rates define subtypes of SAD ~~(consistent vs. variable, which level of airway is involved, and what percent of these airways are involved).~~ We additionally compared our SAD prevalence with literature findings (Supplemental Table 7), yet no study compared all types of physiologic SAD methods. Anderson et al.[6] used R5-R20 >0.03 kPa/L/s as cut-off for abnormality, concluding that abnormal R5-R20 values were present in all severities of asthma, i.e. 65% in British Thoracic Society step2, 64% in step3 and 70% in step4. Our overall prevalence with this cut-off was 70%; we extend their findings showing that prevalence rates of R5-R20 >LLN increase from GINA steps 1-5, being 54%, 65%, 70%, 77%, and 91% respectively. In

contrast, the prevalence of Sacin >LLN was lowest in GINA1, almost identical in GINA 2-4 and highest in GINA5, suggesting that mostly peripheral airway dysfunction, and likely structural changes, are present in most severe asthma. In summary, our data are comparable with published findings in smaller samples, yet expand these observations by providing information on all different SAD measurements at the same time in one group of asthma patients across all severities.

Strengths of our study are the large group of asthma patients covering the full severity spectrum and the extensive work-up and quality and experience of the centers. ATLANTIS is a multi-center international study, therefore we feel our results are reliable and applicable to multiple populations. We also included smokers, a factor that by itself may induce some SAD. We felt it important that our study reflects the larger asthma and non-asthma population globally for generalizability, and thus not restricts the impact of our findings. The controls had comparable age, sex ratio and particularly smoking habits as the asthma population, which provided novel LLN and ULN values for physiological parameters infrequently studied, like IOS and MBNW. We acknowledge that a larger control group might have improved precision of these values, which will be partially overcome when we add the longitudinal data in the future.

We recognize that a quality check of the maneuver to get optimal phase III slope in the MBNW test[27] is key to validity of the measurements, which we have carefully ensured in the present study. The finding that some Sacin values were in the normal range does not contrast with the presence of airway dysfunction in Group1, as the body of the available literature on ventilation heterogeneity in adult asthma[21,28-34] reveals a variable contribution of conducting versus acinar lung regions to treatment response, and consistency in the reversibility towards normal values after exacerbations[28]. Particularly, the persistent derangement of ventilation in conducting airways (Scond) seems more related to airway remodeling, exacerbations, and hyperresponsiveness, whereas the reversible derangement in acinar airway ventilation (Sacin) mainly reflects asthma severity[35]. Accordingly, the

worst clinical SAD score was present in the group with the poorest asthma control and higher prevalence in GINA 4 and 5.

Another limitation is that CT scans were not available in all participants, limiting numbers for analyses. However, this allowed us to demonstrate that the clinical SAD model in the full asthma cohort could be replicated in the smaller group with CTs. Future work will expand our analyses by performing parametric response mapping (PRM)[36], a CT voxel-based imaging biomarker tool to quantify 'functional small airways disease'. A potential limitation is that age was somewhat higher in the asthma than control participants, yet the difference small (median age (interquartile ranges) of 46 (34-54) vs 41 (29-52) years respectively) and likely not of clinical significance, and we adjusted for age in all analyses. We cannot put our clinical SAD score forward as a clinically applicable tool as yet, since this is a cross-sectional analysis. The score already significantly associates with number of exacerbations, asthma severity and control, and the longitudinal phase of the study will elucidate whether it also predicts future changes in these clinical outcomes. For the same reason we cannot put the "best parameters" of SAD forward yet . Additionally, a Small Airways Dysfunction Tool (SADT) will be developed, a questionnaire as an easy measure to suggest SAD[9], which may be easily applicable in the clinic as MBNW and body plethysmography are not available for all routine settings. Our article did not report on SAD with regard to the underlying pathology[9]. However, we will assess which direct and indirect measures of inflammation best discriminate between large and small airways' compartments, with bronchial and transbronchial biopsies in a smaller subset of participants in the future.

Large and small airways obstruction are important components of asthma pathophysiology[1-3]. Our focus is on the small airways and their specific impact upon asthma symptoms and exacerbations, an area of investigation that has been relatively neglected in our opinion (an overview of relatively

small-sized studies is presented in Supplemental Table 7). It would be of interest to analyze in the future subgroups with Large Airway Dysfunction (LAD) without SAD, or conversely, with SAD and without LAD. Finally, one would like to have a 'gold standard' for SAD, yet our study shows this is not feasible since many physiological parameters contribute to the SAD model. This likely reflects that they represent abnormalities in distinct parts of the bronchial tree and/or contrasting aspects of underlying mechanisms of SAD, thereby providing different information[9].

We were able to define a SAD score that reflects the amount of physiological small airways impairment and is significantly associated with asthma control, exacerbations and severity. We additionally observed two clinical SAD Groups that are comparable in e.g. gender, atopy, FeNO, ICS dose and smoking habits, while Group2 was somewhat older, had a longer asthma duration and more severe asthma according to all parameters tested. Interestingly, Sacin)[32], which reflects dysfunction of the most peripheral small airways, was in the normal range in Group1 only and had a higher prevalence in Group2. The difference between the two clinical SAD groups was particularly clear with SAD measurements like IOS and spirometry (Figure 3). Clinical SAD Group 2 represents "more severe" SAD, given particularly the presence of more severe small-to-mid-sized airway obstruction (R5-R20, FEF_{25-75}) and less airway distensibility (AX). In summary, we can detect asthma subtypes based on presence and extent of SAD measured with easy-to-conduct, clinically applicable tools.

Similarly, with regard to the clinical SAD score, we developed a CT-SAD score. The CT-SAD score significantly associated with GINA severity, but less well than the clinical SAD score. CT SAD Group2 had more severe asthma and the physiologic parameters were significantly different from controls and from Group1. However, the CT SAD Groups had similar levels of small-to-mid-sized airway obstruction (R5-R20) and conducting airway ventilation heterogeneity (Scond), reflective of dysfunction in small-medium size conducting airways, while Group2 had significantly higher air trapping (RV/TLC) and acinar airway ventilation heterogeneity (Sacin) values, reflective

of the most peripheral small airways. This suggests that CT scan-derived SAD captures regional differences in mechanisms of airway dysfunction due to air trapping as a surrogate for peripheral airways impairment. They become apparent in supine position, when airway closure and compliance reduction develop as consequence of severe hyperinflation and expiratory reserve volume reduction[37] in more severe asthma. Notably, we observed a difference in airway distensibility (AX) in participants undergoing CT scan, in comparison to those who did not (Supplemental Table 3). It is thus understandable that the Clinical SAD score and the CT SAD score were not concordant (r=0.28). Where CT scans (performed in supine position) provide information on SAD particularly by changes driven from increased residual static lung volumes and air trapping[38], the physiologic parameters measured in the sitting position provide information on air trapping (body plethysmography RV/TLC), small airway obstruction (IOS and FEF_{25-75}) and heterogeneity of both conducting and acinar airway ventilation (MBNW). This potentially explains why the CT SAD score, in contrast to the clinical SAD score, did not associate with health status or asthma control.

Asthma control is lacking in 50-60% of patients despite guideline-based management[39] and untreated SAD has been proposed as a contributing factor[1]. Drivers of asthma control include treatment adherence and appropriate use of inhalers, psychological factors and environmental trigger exposures. The current study suggests that asthma control is also determined by the presence of SAD, since ACT was significantly associated with the clinical SAD score and specifically abnormal in clinical SAD Group2 (most severe SAD). Moreover, a lower ACT score was associated with higher IOS parameters R5 and AX values. Hence asthma control may be partially driven by SAD, but also obstruction in larger airways given its association with FEV_1, the gold standard for diagnosis and severity in clinical practice.

Of interest, asthma participants had higher blood pressure than our controls. We did not find literature reporting this observation. Comorbidities are thus not only present in COPD, another obstructive pulmonary disease [40,41], but also occur in asthma patients with a median age of 46 (34;54) years. This is in agreement with previous studies indicating systemic inflammation as one underlying mechanism linking reduced lung function to cardiovascular mortality[42] and a positive association between lower FEV_1 and systemic arterial hypertension, while lower ICS doses attenuated the likelihood for hypertension in a population with comparable age to ours[43]. Alternatively, hyperinflation could have a role via its contribution to changes in intrathoracic pressure that increase left ventricular wall stress, similar to reports in COPD[44].

In conclusion, our data in a large asthma population covering the full spectrum of asthma severity show the complexity of SAD. Notwithstanding this, the clinical classification of Small Airways Dysfunction is meaningful given its association with asthma severity, control and exacerbations. Results show that SAD can be present across all GINA severity stages. Depending on the type of physiologic parameter used, the prevalence rate changes considerably, but is consistently highest in GINA5. SAD prevalence rates were lowest with Sacin, reflecting pre-acinar/acinar airway abnormalities, and this prevalence was quite comparable over GINA2-4 but again highest in GINA5, suggesting structural abnormalities in severe asthma. In contrast, other physiologic parameters showed either increasing prevalence rates with severity (RV/TLC) or a stepwise increase (FEF_{25-75}, R5-R20, AX, X5). Clinical SAD and CT SAD scores did not significantly correlate. SAD derived from the CT scan provides particularly data on air trapping and ventilation impairment in more peripheral airways, while the physiologic measures show results from both small-medium size conducting airways and peripheral airways. For clinical practice it is important that physiological, easy-to-conduct measures like IOS and spirometry, delineate two asthma SAD subtypes that differ in exacerbation rates, quality of life, asthma severity and control.

Contributors

DP, MK, CB, MvdB, LF, AP, TvdM, KR, SS and DS designed the study. DP, MK, CB, MvdB, LF, GA, AP, TvdM, KR, SS, NG and DS discussed and interpreted the data. The first draft of the paper was written by DP, CB and MK; DP collated input from all co-authors who reviewed all versions of the manuscript. DP and MK had access to raw data. The corresponding author had full access to all of the data and the final responsibility to submit the initial and revised manuscript.

Declaration of interests

D.P. reports that the University of Groningen has received money regarding a research grant from Astra Zeneca, Chiesi, Genentec, GSK and Roche, regarding consultancies from Astra Zeneca, Chiesi, and GSK, outside the submitted work. CB reports grants and personal fees from Chiesi, grants from AirPROM, during the conduct of the study; grants and personal fees from GlaxoSmithKline, AstraZeneca/Medimmune, Boehringer Ingelheim, Novartis, Chiesi, Roche/Genentech, personal fees from Vectura, Theravance, PreP, Gilead, Sanofi/Regeneron Teva, grants from Pfizer and Mologic, personal fees from Gossamer and 4DPharma, outside the submitted work. SB reports personal fees from Chiesi SAS FRANCE, during the conduct of the study; personal fees from employment, outside the submitted work. MvdB reports research grants paid to University from Chiesi, Teva Pharma, GlaxoSmithKline, outside the submitted work. LF reports personal fees, non-financial support and other from Chiesi Farmaceutici, during the conduct of the study; grants, personal fees and non-financial support from Boehringer Ingelheim, Chiesi Farmaceutici, GlaxoSmithKline, Merck Sharp & Dohme, Takeda, AstraZeneca, Novartis,Menarini; personal fees and non-financial support from Pearl Therapeutics and Mundipharma ,personal fees from Zambon, outside the submitted work. AG and GN report employment by Chiesi Farmaceutici S.p.A. which sponsored the study. AP reports grants and personal fees from Chiesi Pharmaceuticals, during the conduct of the study; grants, personal fees, non-financial support and other from Chiesi, Astrazeneca, GlaxoSmithKline, Boehringer Ingelheim, Mundipharma and

personal fees and non-financial support from Menarini, Pfizer, Novartis, Zambon, outside the submitted work. TvdM reports grants and personal fees from Astra Zeneca, TEVA, GSK, personal fees from Boehringer Ingelheim, outside the submitted work; and From 1 June 2017 T van der Molen is a part-time employee of GSK. KR reports personal fees from AstraZeneca, Boehringer Ingelheim, Novartis, Sanofi, Teva, Intermune, Chiesi Pharmaceuticals, Berlin Chemie and grants from Ministry of Education and Science, Germany outside the submitted work. SS reports grants from Chiesi onulus foundation, Sir Jule Thorne Trust, Medical Research Council/EPSRC NAPP, NIHR UK, and personal fees from AZ, GSK, Boehringer Ingelheim, Novartis, Mundipharma, Owlstone, outside the submitted work. D Singh reports grants and personal fees from Chiesi, AstraZeneca, Boehringer Ingleheim, GlaxoSmithKline, Glenmark, Merck, Menarini, Mundipharma, Novartis, Peptinnovate, Pfizer , Pulmatrix, and personal fees from Cipla, Apellis, Genentech, Skyepharma, Teva, Therevance, and Verona, outside the submitted work. MK reports grants from NIH, Chiesi, Sanofi, personal fees from Elsevier, during the conduct of the study.

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Figures and Tables

Figure 1. Flow-diagram of patients and controls without airway obstruction, with reasons for drop out

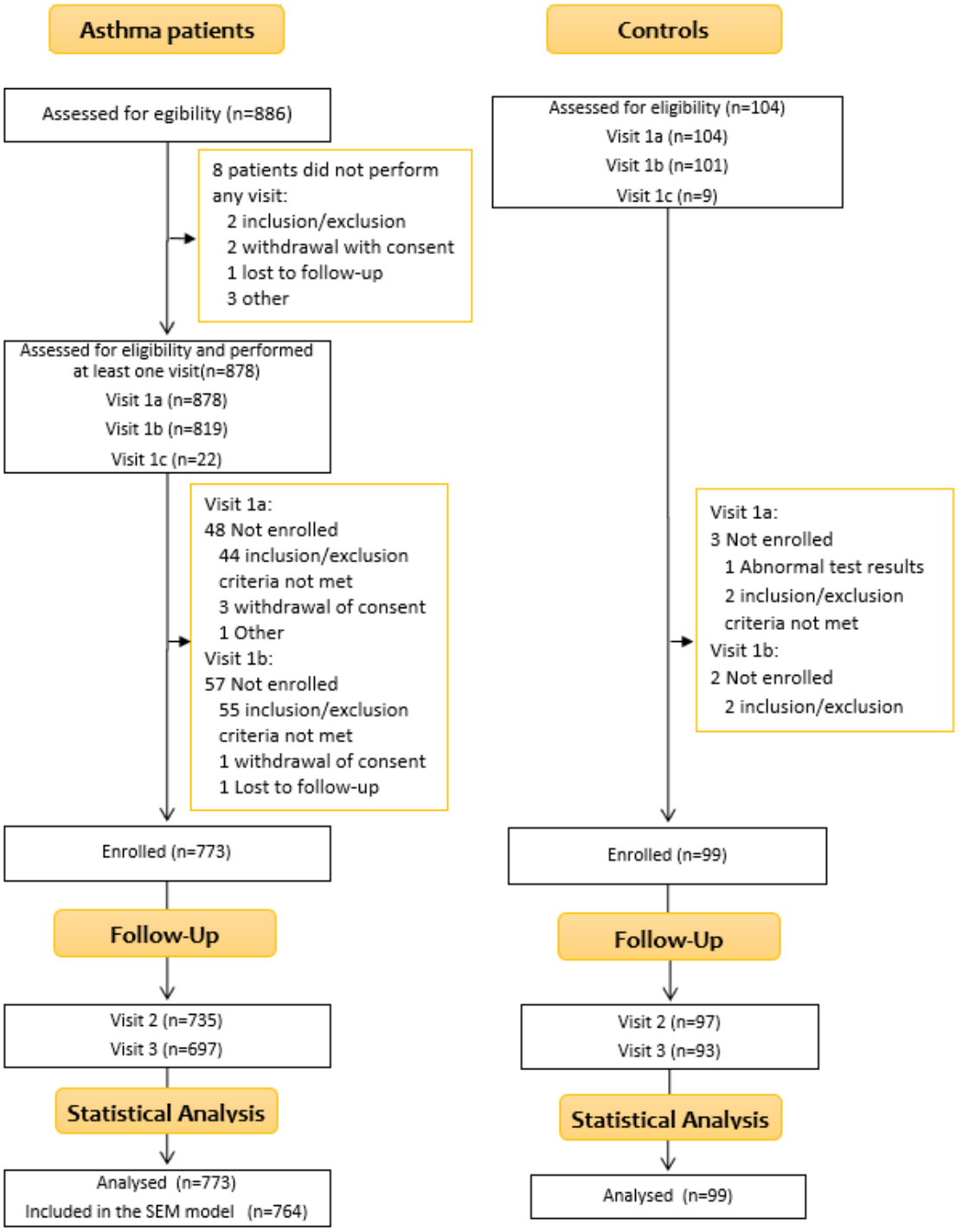
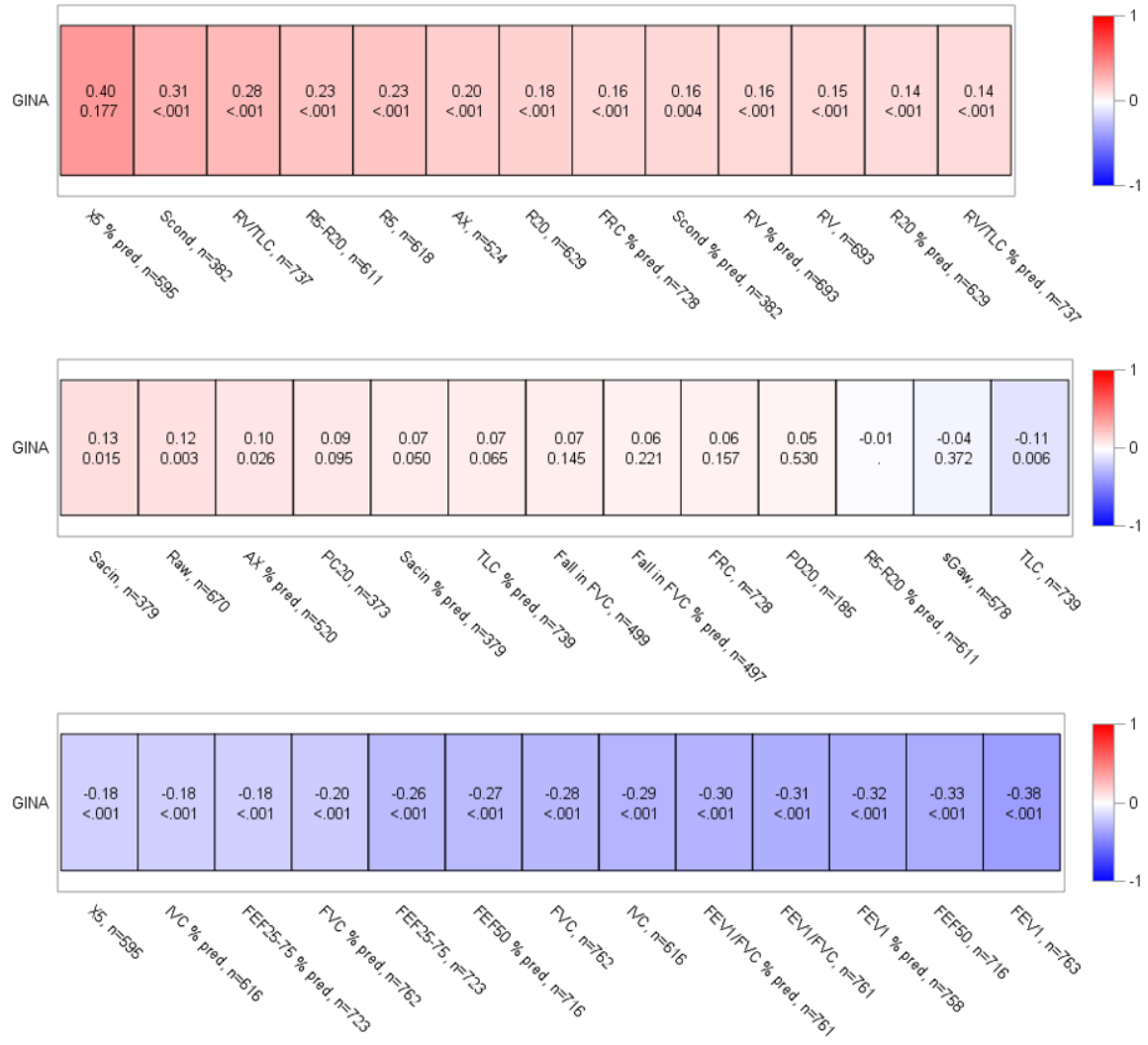
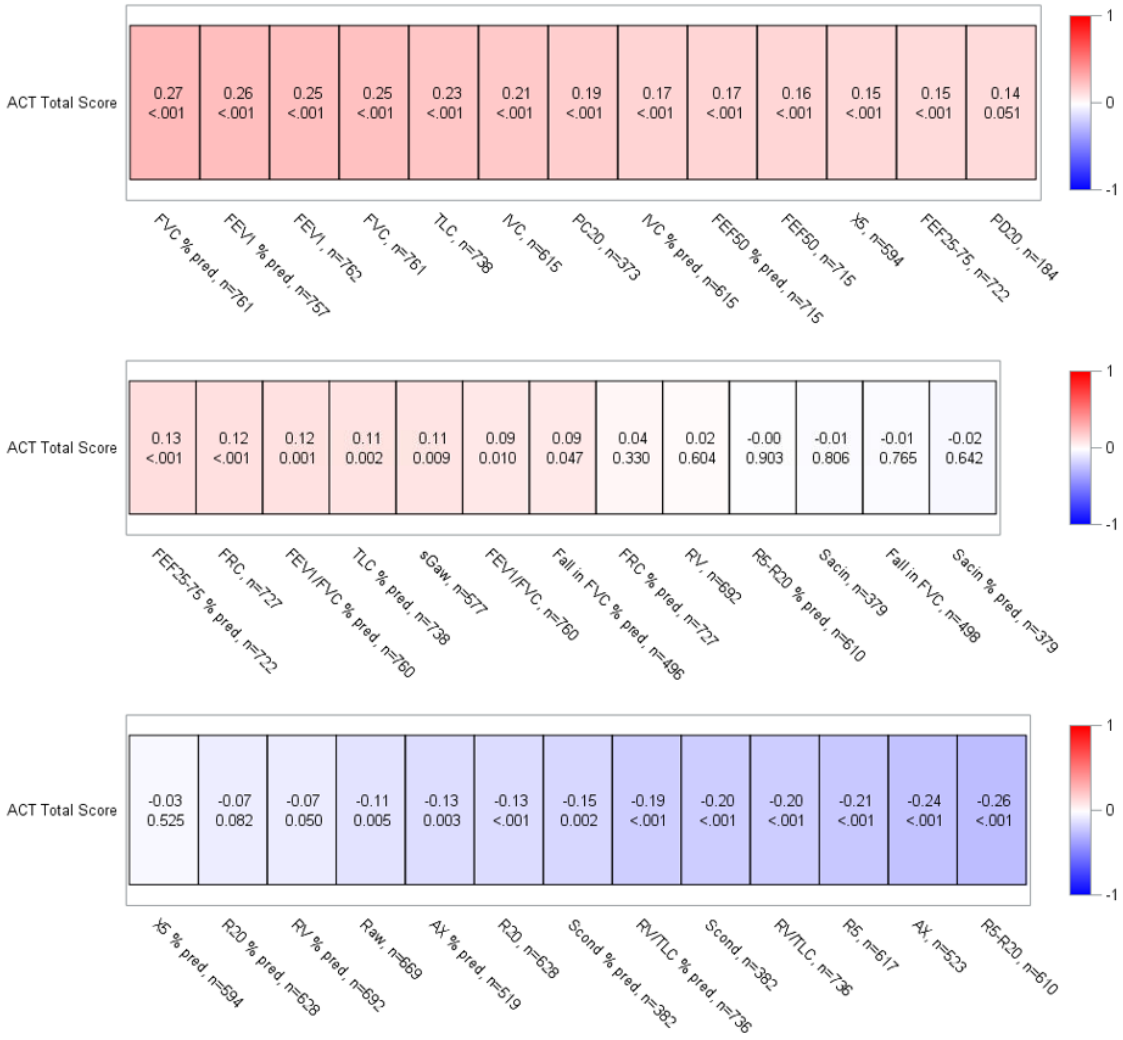


Figure 2. Monovariate correlations of physiological parameters and GINA severity, ACT score and number of exacerbations




ACT Total Score
0.27 <.001
0.26 <.001
0.25 <.001
0.25 <.001
0.23 <.001
0.21 <.001
0.19 <.001
0.17 <.001
0.17 <.001
0.16 <.001
0.15 <.001
0.15 <.001
0.14 0.051
FVC % pred, n=761
FEV1 % pred, n=757
FEV1, n=762
FVC, n=761
TLC, n=738
IVC, n=615
PiC20, n=373
IVC % pred, n=615
FEF50 % pred, n=715
FEF50, n=715
X5, n=594
FEF25-75, n=722
PiD20, n=184
1
0
-1
ACT Total Score
0.13 <.001
0.12 <.001
0.12 0.001
0.11 0.002
0.11 0.009
0.09 0.010
0.09 0.047
0.04 0.330
0.02 0.604
-0.00 0.903
-0.01 0.806
-0.01 0.765
-0.02 0.642
FEF25-75 % pred, n=722
FRC, n=727
FEV1/FVC % pred, n=760
TLC % pred, n=738
sGaw, n=577
FEV1/FVC, n=760
Fall in FVC % pred, n=496
FRC % pred, n=727
RV, n=692
R5-R20 % pred, n=610
Sacin, n=379
Fall in FVC, n=498
Sacin % pred, n=379
1
0
-1
ACT Total Score
-0.03 0.525
-0.07 0.082
-0.07 0.050
-0.11 0.005
-0.13 0.003
-0.13 <.001
-0.15 0.002
-0.19 <.001
-0.20 <.001
-0.20 <.001
-0.21 <.001
-0.24 <.001
-0.26 <.001
X5 % pred, n=594
R20 % pred, n=638
RV % pred, n=692
Raw, n=669
AX % pred, n=519
R20, n=638
Scond % pred, n=382
RV/TLC % pred, n=736
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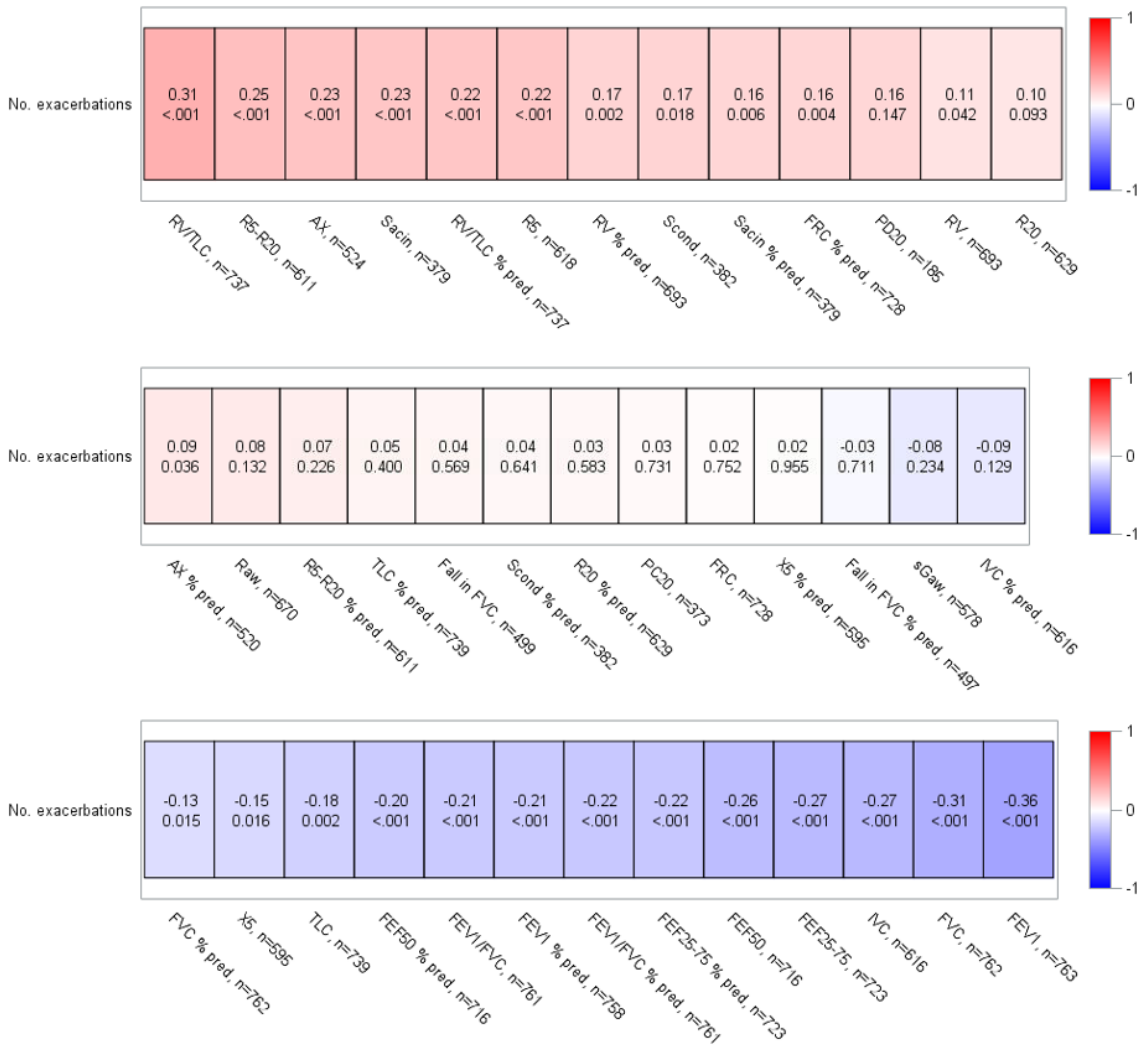
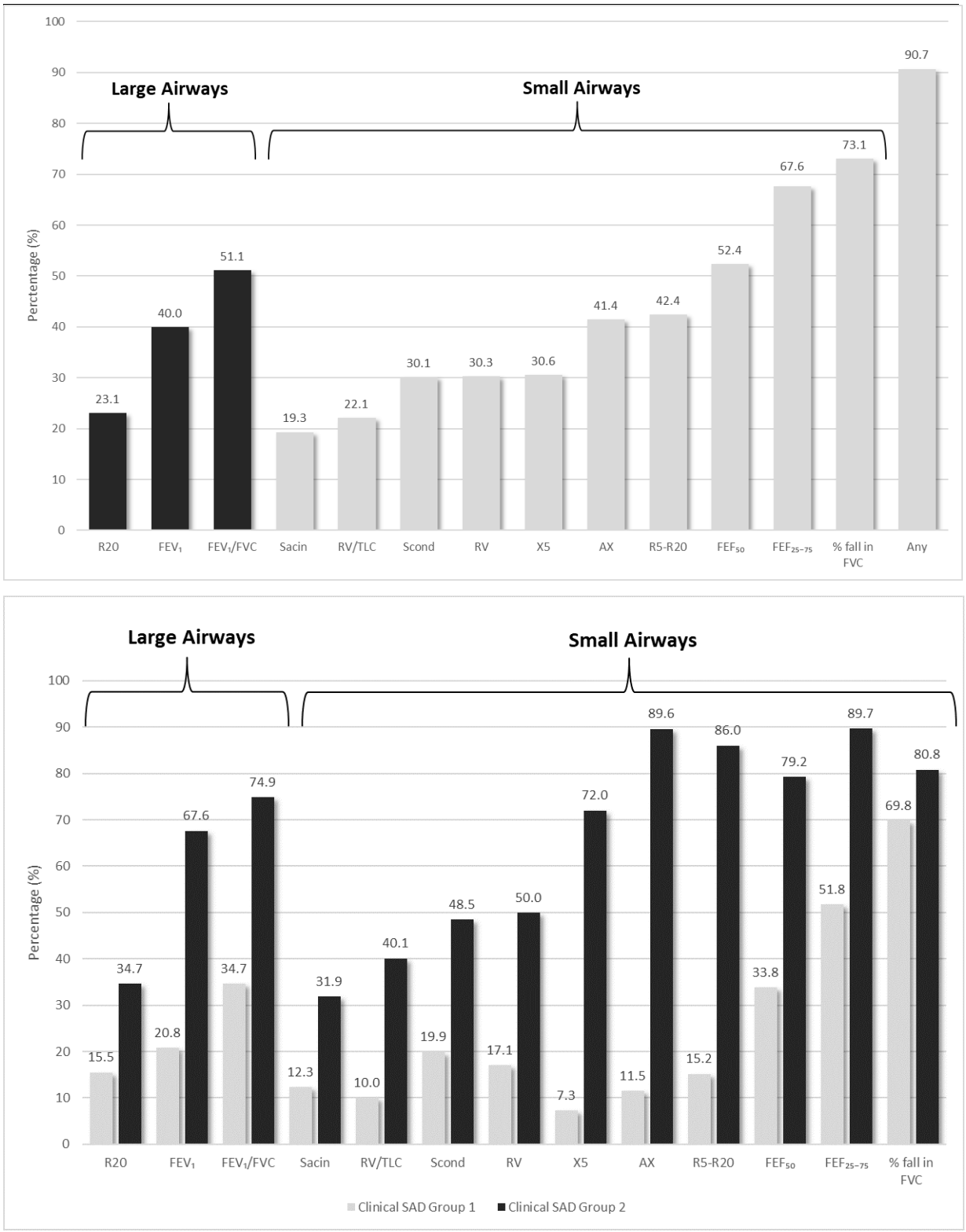
RV/TLC, n=736
R5, n=617
AX, n=523
R5-R20, n=610
1
0
-1



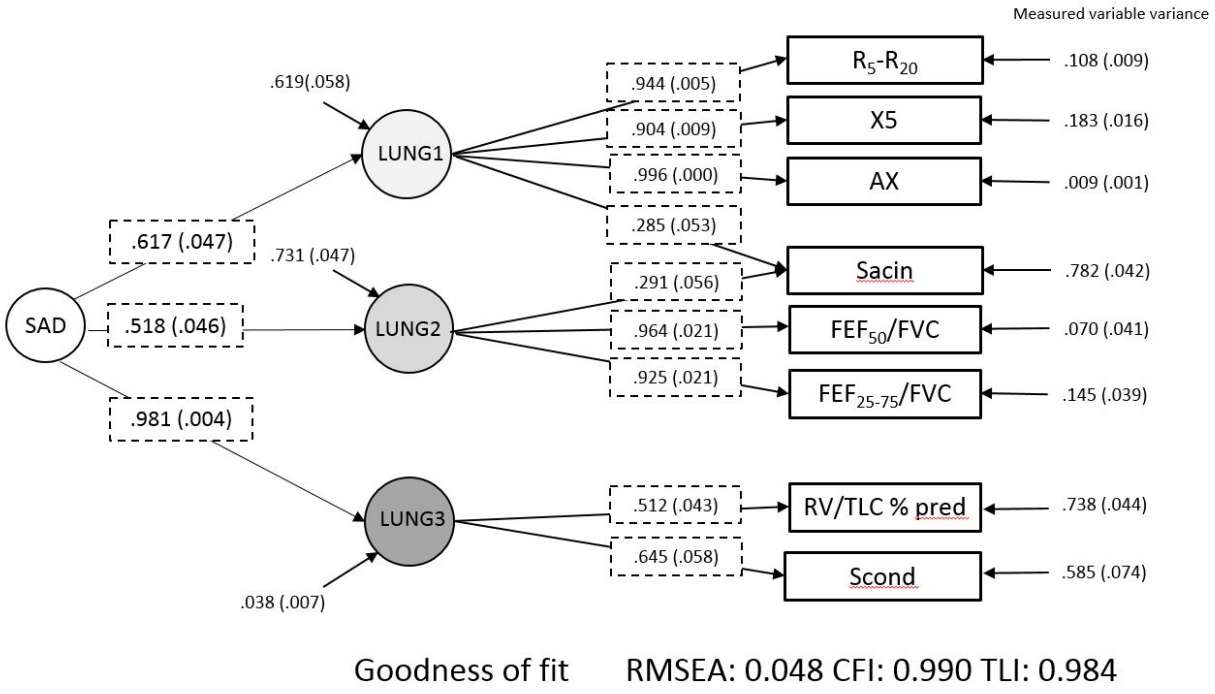
Legend to Figure 2. Correlations are presented for GINA severity (top panel), ACT score (middle panel), and Number of exacerbations in the past year (lowest panel). Darkest red is highest positive correlation between parameters. Darkest blue is the lowest negative correlation between parameters. All abbreviations are presented in Table 1.

Figure 3. Prevalence rates of airways dysfunction in the full asthma cohort and in the 2 SAD subgroups



Legend to Figure 3. Prevalence rates of Large Airways abnormalities, and Small Airways abnormalities in the full cohort of asthma participants (upper Figure), and according to Clinical SAD Group1 and Group2 (lower Figure). Prevalences are based on LLN (Lower Limit of Normal) and ULN (Upper Limit of Normal) values derived from the literature or from ATLANTIS controls without airway disease, noted with*. For abbreviations see Table 2.

Figure 4. Cross-Sectional Clinical SEM analyses of small airway function

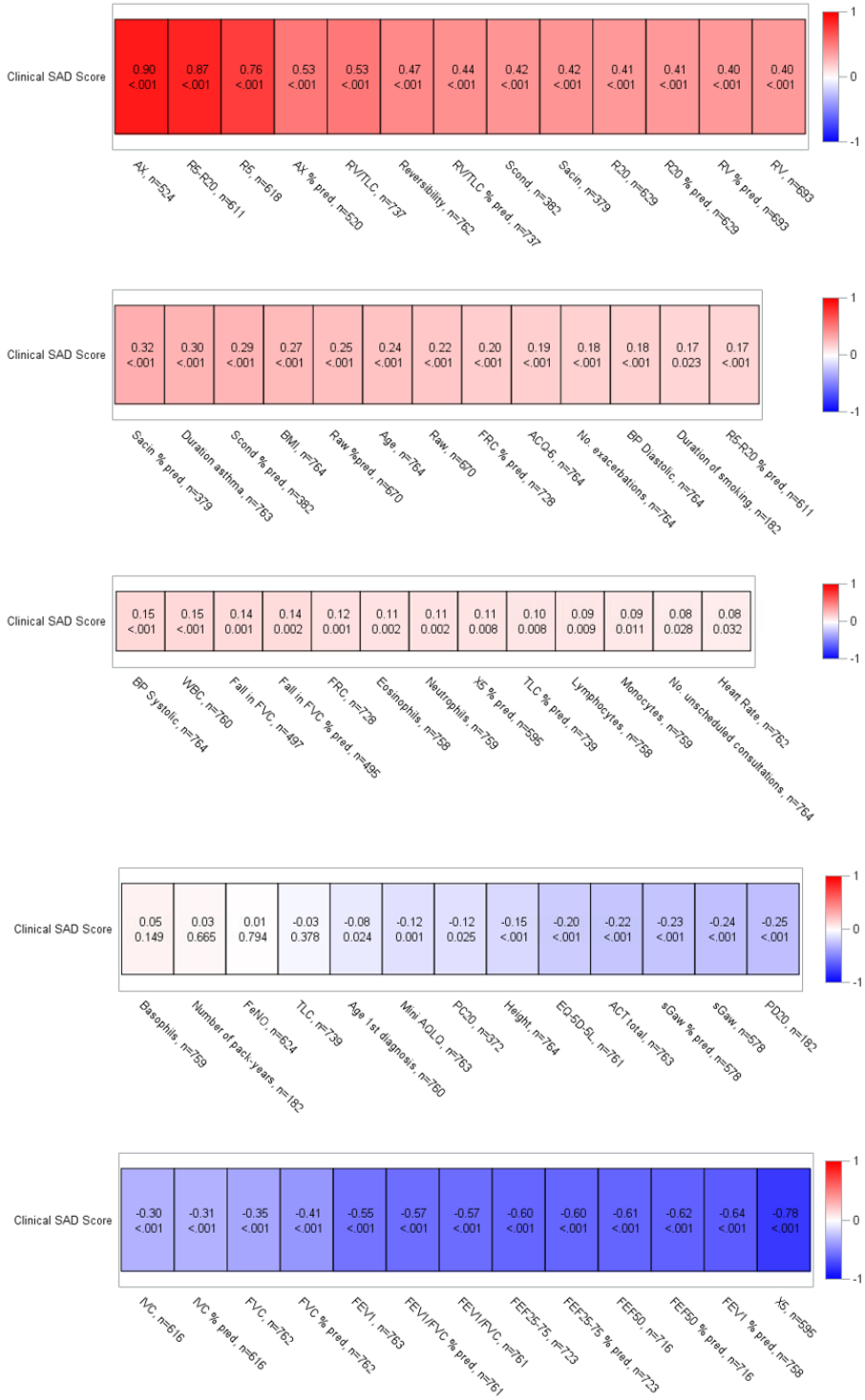


Legend to Figure 4. SAD=Small Airway Dysfunction.

The figure shows the results of Structural Equation Modeling (SEM). The model uses the measured variables presented in squares to define the three latent variables (Lung1, Lung2 and Lung3). The strength of the relationship of each measured variable to the underlying factor is expressed by the factor loading, presented in the Figure in dashed squares. Moreover, the numbers that are not presented in squares are the measured variable variances. The variable SAD is then constructed by a structural model that imputes the relations between these three latent variables (Lung1 loading 0.617, Lung2 loading 0.518 and Lung3 loading 0.981). Thus SEM modeling showed that SAD was built up by three latent variables, represented in circles (Lung1 loading 0.617, Lung2 loading 0.518 and Lung3 loading 0.981). The measured variables are presented in squares . IOS parameters R5-R20, X5 and AX (reflecting small-to-mid-sized airway obstruction/distensibility) loaded to the first latent variable (Lung1), FEF_{50} and FEF_{25-75} both corrected for FVC (reflecting small-to-mid-sized airway obstruction), to the second latent variable, while MBNW parameter Sacin (reflecting dysfunction in the most peripheral airways) loaded both to the first and second latent variable. The lung volume parameter RV/TLC % predicted (most peripheral airways dysfunction) and MBNW parameter Scond (dysfunction in small-medium size conducting airways) loaded to the third latent variable (Lung3). Please Note that Sacin loaded equally with 0.285 and 0.291 to latent variable Lung1 and Lung2 respectively. Please Note that Sacin loaded equally with 0.285 and 0.291 to latent variable Lung1 and Lung2 respectively. The numbers on the right hand

side represent the variance of the measures, i.e. variance in AX is 0.009, contrasting with the variance in RV/TLC % predicted being 0.738. Goodness of fit of the SEM model was evaluated through the following fit indices: Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), Tucker-Lewis Index (TLI). The closer CFI and TLI are to 1 and the closer RMSEA is to 0 the better is the model fit. The goodness of fit values (Supplemental methods) show there is good coherence of this model to SAD. Fall in FVC during hyperresponsiveness testing contributed to the model when analyzed in the subgroup of asthmatic participants who had undergone hyperresponsiveness testing (see also Supplement for model comparison).

Figure 5. Correlations of the Clinical SAD score of asthma participants with all parameters measured



Legend to Figure 5. For abbreviations see Table 2

Table 1. Parameters of SAD as presented in the SEM analyses of the study and their hypothetical location

Physiologic parameters	Interpretation
Spirometry	
FEF_{25-75} (corrected for FVC), L/s/L [13] and FEF_{50} (corrected for FVC), L/s/L [13]	FEFs at 25-75% interval, or at 50% of expired lung volumes are measurements of airflow obstruction in small-to-mid-caliber airways taken at low/mid expiratory lung volumes. When corrected for FVC, they are surrogate measures of the sizes of small-to-mid caliber airways relative to lung size, called dysanapsis. Dysanapsis is a characteristic favoring airways hyperresponsiveness.
Body plethysmography	
RV/TLC ratio, L/L [14]	Air trapping due to obstruction in both conducting small and peripheral airways
FRC, L [14]	Respiratory system resting volume as main determinant of whole airway static dimensions, and airway hysteresis
IOS	
R5-R20, kPa/L/s [15]	Respiratory Resistance of small-to-mid-sized conductive and peripheral airways
X5, kPa/L/s [15]	Respiratory System Reactance reflecting inertance and elasticity (capacitance), including small peripheral airways
AX, kPa/L [15]	Distensibility of the peripheral lungs (parenchyma + small peripheral airways)
MBNW	
Scond*VT, L^{-1} [16]	Index of convectional ventilation heterogeneity in peripheral conducting airways
Sacin*VT, L^{-1} [17]	Index of diffusive ventilation heterogeneity in most peripheral pre-acinar/acinar airways
Hyperresponsiveness	
Fall in FVC at PC_{20} or $PD_{20,}$ % [18,19]	Air trapping due to excessive bronchoconstriction or closure of small airways
CT scan parameters	
MLD ratio, E/I [25]	Ratio of mean lung density for inspiratory versus expiratory scans- a measure of air-trapping due to lung parenchyma inspiratory distension in the supine position
Lung volume ratio, cm^3 [22]	Ratio of CT-derived lung volume for inspiratory versus expiratory scans- a measure of air-trapping due to obstruction in both conducting small and peripheral airways in the supine position
VI-856, HU [21]	The voxel index < -856 Hounsfield Units from the expiratory scans, an index of expiratory air trapping

Legend to Table 1. Numbers in superscript refer to references used. IOS= impulse oscillometry; MBNW= Multiple breath nitrogen washout; CT= computed tomography, HU=Hounsfield Units

Table 2: Baseline clinical, physiologic and CT characteristics of asthma participants and controls without airway disease

Parameter	**Asthma**	**Controls**	**P - value**
	n=773	n=99	
Clinical characteristics			
Age, years	46 (34 ; 54)	41 (29 ; 52)	0.01
Gender, female N (%)	450 (58)	56 (57)	0.75
Heart rate, bpm	71 (65 ; 78)	68 (61 ; 75)	0.01
BP syst, mmHg	123 (114 ; 131)	120 (110 ; 130)	0.01
BP diast, mmHg	80 (70 ; 84)	75 (68 ; 83)	0.06
BMI, kg/m^2	26 (23 ; 30)	24 (21 ; 27)	<0.001
Atopy (Phadiatop), N (%)	454 (81)	39 (46)	<0.001
FeNO, ppb	25 (16 ; 38)	18 (11 ; 26)	<0.001
Ex-smoker, N (%)	156 (20)	19 (19)	0.39
Current Smoker, N (%)	27 (4)	1(1)	
Eosinophils, 10^9 /L	0.2 (0.1 ; 0.4)	0.1 (0.1 ; 0.2)	<0.001
Neutrophils, 10^9 /L	3.7 (3.0 ; 4.7)	3.3 (2.7 ; 4.4)	0.01
PC_{20}, mg/mL	1.25 (0.4 ; 4.2)	15.23 (16.0 ; 16.0)	<0.001
PD_{20}, mg	0.11 (0.0 ; 0.6)	1.86 (2.0 ; 2.0)	<0.001
Moderate-severe hyperresponsiveness, N (%)	271 (48.4)	0 (0.0)	<0.001
Fall in FVC, %	17 (12 ; 22)	4 (1 ; 8)	<0.001
Lung Physiology characteristics (%predicted)			
FEV_1, %predicted	82.7 (69.9 ; 93.8)	100.4 (91.6 ; 107.3)	<0.001
Change FEV_1, %predicted	7.6 (4.1 ; 12.7)		
FEV_1/FVC, %predicted	85.8 (76.5 ; 93.9)	98.2 (93.8 ; 102.7)	<0.001
IVC, %predicted	99.0 (18.21)	109.7 (15.28)	<0.001
FEF_{50}, %predicted	62.0 (43.2 ; 84.1)	102.0 (84.8 ; 117.3)	<0.001
FEF_{25-75}, %predicted	56.6 (37.6 ; 75.6)	90.7 (75.6 ; 108.1)	<0.001

RV, %predicted	117.1 (98.4 ; 138.9)	95.6 (87.0 ; 115.7)	<0.001
TLC, %predicted	104.9 (95.9 ; 115.5)	104.8 (96.7 ; 112.5)	0.62
RV/TLC, %predicted	106.1 (91.6 ; 125.8)	92.5 (80.6 ; 109.6)	<0.001
FRC, %predicted	108.7 (93.4 ; 126.7)	107.6 (91.9 ; 121.4)	0.42
Raw, %predicted	143.0 (91.4 ; 231.1)	77.6 (62.9 ; 99.5)	<0.001
sGaw, %predicted	60.5 (42.5 ; 94.7)	85.0 (61.3 ; 124.6)	<0.001
R20, %predicted	114.6 (97.4 ; 134.9)	96.5 (84.7 ; 110.2)	<0.001
R5-R20, %predicted	278.6 (91.2 ; 640.9)	69.5 (0.0 ; 161.7)	<0.001
X5, %predicted	130.4 (94.4 ; 184.7)	94.6 (77.6 ; 119.7)	<0.001
AX, %predicted	209.3 (95.0 ; 510.0)	66.1 (49.9 ; 108.0)	<0.001
Scond*VT, %predicted	180.5 (100.7 ; 305.3)	95.6 (44.8 ; 149.6)	<0.001
Sacin*VT, %predicted	107.2 (76.7 ; 154.8)	94.1 (61.6 ; 129.8)	0.01
CT Scan characteristics			
MLD Inspiratory, HU	-837.93 (-856.95 ; -811.97)	-839.89 (-853.81 ; -812.76)	0.65
MLD Ratio E/I	0.83 (0.77 ; 0.88)	0.80 (0.73 ; 0.87)	0.08
VI-856	7.82 (2.5; 19.5)	7.83 (1.5; 15.5)	0.35
Lung Volume Ratio	0.50 (0.43 ; 0.60)	0.47 (0.38 ; 0.56)	0.16
Percentile 15 Inspiratory	-921 (-935;-904)	-929 (-940;-899)	0.46
Median LA/BSA Inspiratory	10.4 (2.93)	11.4 (2.83)	0.03
Median LA Inspiratory	19.0 (15.7 ; 23.3)	21.3 (18.5 ; 25.6)	0.01
Pi10 Inspiratory	7.21 (6.59 ; 7.77)	6.70 (6.28 ; 7.84)	0.07
Po20 %WA Inspiratory	7.41 (6.67 ; 8.50)	7.33 (6.42 ; 9.02)	0.73

Legend to Table 2: All parameters are presented as Mean (standard deviation), Median (Quartile1 - Quartile 3), or N (%) as appropriate. BP= Blood Pressure, Syst=Systolic, BMI= Body Mass Index, FeNO=Fraction of exhaled Nitric Oxide, WBC=White Blood Cell, RV= Residual Volume, FRC=Functional Residual Capacity, PC=Provocative Concentration, PD=Provocative Dose, PC_{20} and PD_{20} = the provocative concentration and dose, respectively, that cause a 20% fall in FEV_1 from baseline FEV_1 during methacholine challenge, Fall in FVC, % fall in FVC at PC_{20} or PD_{20}; FEV_1=Forced Expiratory Volume in the 1st second, FVC= Forced Vital Capacity, FEF_{50}=Forced Expiratory Flow at 50% of FVC, IVC=Inspiratory Vital Capacity, FEF_{25-75}= Forced Expiratory Flow at 25%-75% of FVC,RV= Residual Volume, TLC=Total Lung Capacity, FRC= Functional residual Capacity, Raw- airway resistance, sGaw= specific

airway conductance, R5-R20= Peripheral Airway Resistance, X5= Resistance at 5 Hz, AX= Area of Reactance, Scond*VT= ventilation inhomogeneity in the conductive zone of the lungs, Sacin*VT= Ventilation inhomogeneity of the acinar zone of the lungs, CT= Computed tomography, MLD Ratio E/I= Mean Lung Density Expiratory to Inspiratory ratio, E=Expiratory, I=Inspiratory, LA= Lumen Area (mm^2), BSA= Body Surface Area (m^2), VI-856= Voxel index at -856 Hounsfield Units.

Table 3 Characteristics of asthma participants

Parameter	
GINA 1, N (%)	135 (17.5)
GINA 2, N (%)	85 (11.0)
GINA 3, N (%)	207 (26.8)
GINA 4, N (%)	300 (38.8)
GINA 5, N (%)	46 (6.0)
Medication use	
SABA, N (%)	671 (86.8)
Short acting anticholinergics, N (%)	9 (1.2)
LABA, N (%)	86 (11.1)
ICS, uncombined N (%),	183 (23.9)
Extra-fine ICS, N (%)	58 (7.5)
Non-extra-fine ICS, N (%)	127 (16.4)
ICS mean daily dose (BDP equivalent), µg	669 (446)
ICS/LABA, N (%)	460 (59.5)
ICS/LABA mean daily dose (BDP-equivalent), µg	882 (634)
Extra-fine ICS/LABA, N (%)	124 (16.0)
Non-extra-fine ICS/LABA, N (%)	336 (43.5)
Oral corticosteroids, N (%)	22 (2.8)
Oral corticosteroids mean daily dose, mg	7.5 (5.0 ; 20.0)
Montelukast, N (%)	144 (18.6)
LAMA, N (%)	29 (3.8)
Biologics, N (%)	32 (4.1)
Duration of disease, years	16.7 (5.6 ; 29.3)
Age 1st diagnosis <18 years, %	39
Unscheduled consultations past 12 months, N	0.3 (1.4)
Exacerbations past 12 months, N	0.2 (0.6)
>1 exacerbation past 12 months, %	14

ACT, total score	21.0 (18.0 ; 24.0)
ACT < 15, %	13
ACQ-6, total score	0.8 (0.3;1.5)
ACQ-6 > 1.25, %	33
EQ-5D-5L, VAS score	80.0 (70.0 ; 90.0)
Mini AQLQ, total score	5.6 (4.7 ; 6.3)

Legend to Table 3. Data are presented as N (%) or Median (Q1 to Q3 ranges) as appropriate. ACT=Asthma Control Questionnaire, ACQ-6= Asthma Control Questionnaire-6, EQ-5D-5L= Standardized measure of health status descriptive system, Mini AQLQ= Mini Asthma Quality of Life Questionnaire. Number of exacerbations and unscheduled consultations are based on the past 12 months. The daily dose of ICS (inhaled corticosteroids) is expressed in BDP equivalents, µg/day

Table 4. Prevalence rates (%) of abnormal SAD parameters (>ULN or <LLN) according to GINA stages

Parameter, %	**GINA 1**	**GINA 2**	**GINA 3**	**GINA 4**	**GINA 5**
FEF_{25-75}	41.4	43.0	50.5	54.5	80.4
FEF_{50}	37.3	49.4	54.1	55.3	75.0
% fall FVC	71.7	67.9	75.2	72.7	84.2
RV/TLC	14.0	16.3	19.3	28.1	31.1
FRC	16.2	23.4	19.1	24.5	27.3
R5-R20	29.9	40.0	36.5	50.5	70.6
AX	32.4	34.4	35.4	49.2	67.7
X5	22.8	31.8	28.5	33.2	53.1
Scond	20.5	20.0	30.0	33.3	63.6
Sacin	12.3	17.8	18.5	20.5	40.9

Legend to Table 3. for abbreviations see Table 2. GINA severity was based on past treatment used. Note that the highest prevalence of SAD is always in GINA5, the lowest prevalence across all GINA stages is with Sacin.

Table 5. Relationship of lung physiology variables with number of exacerbations and unscheduled consultations

Number of exacerbations

Independent variables included in the final model	Coefficient	P-value type 1	P-value type 3
$FEF_{25-75,}$ L/s $_{corrected\ for\ FVC}$	-1.226	0.034	
R5-R20, kPa/L/s	2.894	0.01	
Raw, kPa*s/L	-2.286	0.01	
RV/TLC, ratio	2.773	0.04	
sGaw, 1/kPa*s	-0.316	0.03	
Height, cm	-0.053	<.001	
PC_{20} and PD_{20} categories – very mild vs normal	-1.058	0.02	0.006
PC_{20} and PD_{20} categories - mild vs normal	-1.624	<.001	
PC_{20} and PD_{20} categories - moderate-severe vs normal	-1.212	0.01	
Sex - Female vs Male	0.717	0.03	

Number of unscheduled consultations due to worsening symptoms

Independent variables included in the final model	Coefficient	P-value type 1	P-value type 3
FEV_1 , L	0.647	<.001	
FRC , L	-0.425	0.01	
RV/TLC, ratio	4.659	0.01	
sGaw , 1/kPa*s)	-0.466	<.001	
PC_{20} and PD_{20} categories – very mild vs normal	-0.999	0.01	0.02
PC_{20} and PD_{20} categories - mild vs normal	-0.888	0.01	
PC_{20} and PD_{20} categories - moderate-severe vs normal	-0.792	0.01	
Sex (male/female) - Female vs Male	0.647	0.02	

Legend to Table 4. MBNW parameters were not used, since this would restrict the number of asthmatics to be analyzed (see Methods). P-value type 3 assesses the statistical difference in hyperresponsiveness severity stages. The coefficients are per 1 unit increase in each parameter. As example: the estimate of R5-R20 (kPa/L/s) for exacerbations is 2.894, one

needs to calculate exp(2.894) = 18.065 and this means that for 1-unit increase of R5-R20 the mean number of exacerbations will increase by a factor of 18.07, holding other variables constant. Mild hyperresponsiveness means a higher PD_{20} or PC_{20} value. Patients with more severe hyperresponsiveness have more frequent exacerbations and unscheduled consultations. For abbreviations see Table 2.

Table 6. Clinical characteristics of asthma participants in Clinical SAD Group1 and Clinical SAD Group2

Parameter	Group1 (n=452)	Group2 (n=312)	P-value
Clinical SAD score	-0.256 (-0.34;-0.16)	0.284 (0.12;0.56)	<0.001
Age, years	43 (30;53)	50 (40;58)	<0.001
Gender, female N (%)	257 (57)	186 (60)	0.45
Heart rate, bpm	70 (64;77)	72 (65;80)	0.02
BP syst, mmHg	120 (110;130)	125 (117;135)	<0.001
BP diast, mmHg	78 (70;82)	80 (72;87)	<0.001
BMI, kg/m^2	25 (22;28)	28 (25;32)	<0.001
Atopy, N (%)	262 (81)	187 (79)	0.53
FeNO, ppb	24 (16;37)	25 (16;39)	0.42
Ex-smoking, N (%)	90 (20)	65 (21)	0.47
Duration smoking, years	10 (5.1;16.7)	14 (8.0;20.0)	0.02
GINA 1/2, N (%)	157 (35)	60 (9)	<0.001
GINA 3, N (%)	135 (30)	70 (22)	<0.001
GINA 4/5, N (%)	160 (35)	182 (58)	<0.001
ICS uncombined, N (%)	98 (22)	83 (27)	0.12
ICS/LABA, N (%)	254 (56)	202 (65)	0.02
ICS dose, BDP equivalence	603.2 (384.9)	739.9 (482.5)	0.08
ICS/LABA dose, BDP equivalence	818.8 (563.1)	959.6 (710.8)	0.08
Oral corticosteroids, N (%)	8 (1.8)	14 (4.5)	0.03
Eosinophils, 10^9 /L	0.21 (0.12;0.35)	0.26 (0.16;0.40)	<0.001
Neutrophils, 10^9 /L	3.50(2.88;4.47)	3.90(3.07;4.91)	<0.001
FEV_1, %predicted	90.2 (80.1 ; 98.4)	70.1 (58.8 ; 81.8)	<0.001
Change FEV_1, %predicted	6.5 (3.6 ; 9.9)	10.2 (5.5 ; 14.9)	<0.001
FEV_1/FVC, %predicted	90.1 (83.4 ; 96.6)	78.3 (70.5 ; 86.0)	<0.001
FEF_{50}, %predicted	75.2 (59.1 ; 94.8)	44.4 (31.5 ; 59.7)	<0.001
IVC, %predicted	103.3 (18.0)	93.1 (17.0)	<0.001
FEF_{25-75}, %predicted, N (%)	66.6 (51.7 ; 86.9)	37.7 (27.8 ; 52.2)	<0.001

RV, %predicted	108.9 (92.7 ; 127.2)	134.2 (110.9 ; 158.8)	<0.001
TLC, %predicted	104.3 (95.7 ; 114.0)	105.9 (95.9 ; 116.9)	0.24
FRC, %predicted	107.3 (91.7 ; 123.0)	111.2 (94.8 ; 129.9)	0.01
Raw, %predicted	110.1 (81.4 ; 167.8)	192.3 (139.6 ; 309.3)	<0.001
sGaw, %predicted	66.5 (47.4 ; 105.1)	47.0 (33.9 ; 72.4)	<0.001
R20, %predicted	107.8 (92.2 ; 125.7)	126.3 (109.7 ; 147.9)	<0.001
R5-R20, %predicted	129.6 (29.0 ; 304.0)	636.3 (378.2 ; 1065.0)	<0.001
X5, %predicted	109.1 (80.9 ; 140.5)	199.0 (151.6 ; 254.6)	<0.001
AX, %predicted	115.3 (65.3 ; 198.3)	613.6 (384.7 ; 868.3)	<0.001
Scond*VT, %predicted	144.6 (75.9 ; 239.7)	245.2 (161.7 ; 392.1)	<0.001
Sacin*VT, %predicted	93.1 (70.6 ; 127.0)	140.8 (95.8 ; 190.5)	<0.001
No. unscheduled consultations , N	0.15 (0.57)	0.50 (2.08)	0.001
No. exacerbations, N	0.16 (0.52)	0.29 (0.76)	0.002
>= 1 exacerbation, N (%)	50 (11.1)	59 (18.9)	0.002
Duration of disease, years	11.6 (4.4 ; 24.5)	21.5 (9.4 ; 35.0)	<0.001
Age at 1st **Diagnosis, years**	25 (10 ; 41)	22 (7 ; 41)	0.13
Age at 1st **Diagnosis < 18 years, N(%)**	162 (36.2)	134 (42.9)	0.06
ACT, total score	22.0 (19.0 ; 24.0)	20.0(17.0 ; 23.0)	<0.001
ACT score $\leq$ 15, N (%)	40 (8.9)	60 (19.2)	<0.001
ACQ-6, total mean score	0.66 (0.2 ; 1.3)	1.00 (0.5 ; 1.8)	<0.001
ACQ-6 score $\geq$ 1.25, N (%)	124 (27.4)	126 (40.4)	<0.001
EQ-5D-5L, VAS score	83.0 (75.0 ; 90.0)	80.0 (70.0 ; 90.0)	<0.001
Mini-AQLQ, total score	5.7 (4.8;6.4)	5.5(4.5;6.3)	
CT Scan characteristics			
MLD Inspiratory, HU	-844.53(-859.56 ; -815.71)	-831.65(-854.46 ; -808.68)	0.09
MLD Ratio E/I	0.82 (0.76 ; 0.87)	0.84 (0.78 ; 0.90)	0.01
VI-856	6.96 (1.92 ; 18.27)	9.54 (3.18 ; 21.30)	0.07
Lung Volume Ratio	0.49 (0.41 ; 0.56)	0.51 (0.45 ; 0.62)	0.008
Percentile 15 Inspiratory	-922.33 (-937.51 ; -906.97)	-917.72 (-930.20 ; -900.38)	0.05
Median LA/BSA Inspiratory	10.95 (2.66)	9.67 (3.08)	<0.001

Median LA Inspiratory	20.37 (17.32 ; 23.47)	17.82 (14.59 ; 22.08)	<0.001
Pi10 Inspiratory	7.12 (6.54 ; 7.77)	7.28 (6.59 ; 7.78)	0.64
Po20 %WA Inspiratory	7.49 (6.71 ; 8.52)	7.27 (6.57 ; 8.41)	0.46

Legend to Table 6. Data are presented as N (%), Mean (SD) and Median (interquartile ranges) as appropriate; for abbreviations see Table 2 and Table3.

Exploring the relevance and extent of small airways dysfunction in asthma~~: :~~ baseline data from the AssessmenT of smalL Airways involvemeNT In aSthma~~, the~~ (ATLANTIS) prospective cohort study

Postma DS[1], MD Prof, Brightling C[2], MD Prof, Baldi S[2,11], MD PhD, Van den Berge M[1] , MD, Fabbri LM[3,4], MD Prof, Gagnatelli A[5], PhD, Papi A[6], MD Prof, Van der Molen T[7], MD, Prof, Rabe KF[8], MD Prof, Siddiqui S[2],MD, Singh D[9], MD Prof, Nicolini G[5], PhD, Kraft M[10] , MD Prof, and the ATLANTIS study group*~~Postma DS[1], Brightling C[2], Baldi S[2,11], Van den Berge M[1], Fabbri LM[3,4], Gagnatelli A[5], Papi A[6], Van der Molen T[7], Rabe KF[8], Siddiqui S[2], Singh D[9], Nicolini G[5], Kraft M[10] and the ATLANTIS study group*~~

~~**Collaborators**~~

~~* Badorrek P, Boersma W, Broeders M, Chetta A, Cukier A, D'Amato M, Djukanovic R, Foschino MP, Gessner C, Hanania N, Martin R, Milleri S, Olivenstein R, Paggiaro P Pizzichini E, Plaza Moral V, Scichilone N, Schilz R, Spanevello A, Stelmach R, Vroegop J, Usmani OS, Zhang Q.~~

Author Information

1. University of Groningen, University Medical Center Groningen, Department of Pulmonology, Groningen Research Institute for Asthma and COPD, Groningen, The Netherlands University of Groningen, University Medical Center Groningen, Department of Pulmonology, Groningen Research institute for Asthma and COPD, Groningen, The Netherlands
2. Institute for Lung Health, NIHR Biomedical Research Centre, University of Leicester, UK; Department of Global Clinical Development, Chiesi SAS, Bois Colombes, France
3. Research Centre on Asthma and COPD, University of Ferrara, Ferrara, Italy

4. COPD Centre, Sahlgrenska University Hospital, Gothenburg
5. Department of Global Clinical Development, Chiesi Farmaceutici SpA, Parma, Italy
6. Research Centre on Asthma and COPD, University of Ferrara, Ferrara, Italy
7. University of Groningen, University Medical Center Groningen, Department of General Practice, Groningen Research Institute for Asthma and COPD, Groningen, The Netherlands
8. Lungen Clinic, Grosshansdorf, and Department of Medicine, Christian Albrechts University, Kiel, Germany. Airway Research Center North in the German Center for Lung Research (DZL), Germany.
9. Centre for Respiratory Medicine and Allergy, University of Manchester, University Hospital of South Manchester, Manchester, UK.
10. University of Arizona College of Medicine, Department of Medicine, Tucson, Arizona, United States
11. Department of Global Clinical Development, Chiesi SAS, Bois Colombes, France

Link to full study:

https://clinicaltrials.gov/ct2/show/NCT02123667?term=NCT02123667&rank=1

Summary ~~**Background**~~

~~Small airways dysfunction (SAD) is well-recognized in asthma, yet its role in asthma severity and asthma control is unclear.~~

~~**Methods**~~

~~This multinational observational study investigated participants without and with asthma (GINA severity stage 1-5). They underwent spirometry, body plethysmography, impulse oscillometry (IOS), Multiple Breath Nitrogen Washout (MBNW), computed tomography (CT) and questionnaires. Structural equation modeling (SEM) was applied in asthma to assess the contribution of all physiological and CT parameters to SAD. With SEM, we defined a clinical-SAD and CT-SAD score. Asthma subjects were classified in SAD groups using model-based clustering. Asthma severity, control and health care utilization in the past year were compared with the SAD scores and SAD groups.~~

~~**Findings**~~

~~We investigated 773 asthma and 99 control participants (median [interquartiles] age 46 [34, 54] and 41 [29, 52] years, 58% and 57% females, respectively). All physiologic measures contributed to the clinical SAD model with SEM analysis. The prevalence of SAD in asthma was dependent on the measure used and lowest with MBNW Sacin that reflects ventilation heterogeneity in the most peripheral, pre-acinar/acinar airways. IOS and spirometry, reflecting dysfunction of small-to-mid-sized airways, contributed most to the Clinical-SAD score and differentiated the two SAD Groups. Clinical-SAD Group1 (n=452) had "milder" SAD, i.e. comparable ventilation heterogeneity in pre-acinar/acinar airways values (MBNW Sacin) with controls. Group2 (n=312) had more abnormal physiologic SAD measures than Group1, particularly IOS and spirometry, and more severe asthma (asthma control, treatments, exacerbations, quality of life). Clinical-SAD scores were higher in~~

~~Group2 ("more severe" SAD) and related to asthma control, severity, and exacerbations. Clinical-SAD and CT-SAD scores did not significantly correlate.~~

~~**Interpretation**~~

~~SAD has multiple components and physiologic parameters from spirometry, body plethysmography, IOS and MBNW contribute to SAD. SAD is present across all asthma severity and particularly in severe disease. The clinical classification of SAD in two groups, i.e. a "milder" and "more severe" SAD group, by the easy-to-conduct measures IOS and spirometry, is meaningful given its association with GINA asthma severity stages, asthma control, quality of life, and exacerbations. Further work is needed~~

Background

Small airways dysfunction (SAD) is well-recognized in asthma, yet its role in asthma severity and asthma control is unclear. Our study aimed to assess which (combination of) biomarkers, physiological testing and imaging markers best measures the presence and extent of SAD in asthma.

Methods

This multinational observational study investigated participants without and with asthma (GINA severity stage 1-5). Asthma inclusion criteria were: 1) age 18-65 years; 2) clinical asthma diagnosis > 6 months, confirmed by a chest physician 2, supported by objective evidence of any of the following at the baseline visit or in the previous 5 years: a) positive airway hyperresponsiveness to methacholine, ***or*** b) positive reversibility ($\Delta FEV_1 \geq$ 12% and ≥ 200 mL within 30 minutes after 400 µg of salbutamol pMDI with or without a spacer ***or c)*** PEF variability >20%, measured during 7 days ***or*** d) documented reversibility after a cycle (e.g. 4 weeks) of maintenance anti-asthma treatment; 3) stable asthma on any previous regular asthma treatment ("rescue" β2-agonists alone included) at a stable dose for > 8 weeks before baseline; 4) lifetime smoking ≤ 10 pack-years. They

underwent spirometry, body plethysmography, impulse oscillometry (IOS), Multiple Breath Nitrogen Washout (MBNW), computed tomography (CT) and questionnaires. Structural equation modeling (SEM) was applied in asthma to assess the contribution of all physiological and CT parameters to SAD. With SEM, we defined a clinical-SAD and CT-SAD score. Asthma subjects were classified in SAD groups using model-based clustering. Asthma severity, control and health care utilization in the past year were compared with the SAD scores and SAD groups.

Findings

We investigated 773 asthma and 99 control participants (median [interquartiles] age 46 [34, 54] and 41 [29, 52] years, 58% and 57% females, respectively). All physiologic measures contributed to the clinical SAD model with SEM analysis. The prevalence of SAD in asthma was dependent on the measure used and lowest with MBNW Sacin that reflects ventilation heterogeneity in the most peripheral, pre-acinar/acinar airways. IOS and spirometry, reflecting dysfunction of small-to-mid-sized airways, contributed most to the Clinical-SAD score and differentiated the two SAD Groups. Clinical-SAD Group1 (n=452) had "milder" SAD, i.e. comparable MBNW Sacin with controls. Group2 (n=312) had more abnormal physiologic SAD measures than Group1, particularly IOS and spirometry, and more severe asthma (asthma control, treatments, exacerbations, quality of life). Clinical-SAD scores were higher in Group2 ("more severe" SAD) and related to asthma control, severity, and exacerbations. Clinical-SAD and CT-SAD scores did not significantly correlate.

Interpretation

SAD is a complex and silent signature of asthma, which is likely to be directly or indirectly captured by combinations of physiologic tests: spirometry, body plethysmography, IOS, and MBNW. SAD is present across all asthma severity and particularly in severe disease. The clinical classification of SAD in two groups, i.e. a "milder" and "more severe" SAD group, by the easy-to-conduct measures IOS and spirometry, is meaningful given its association with GINA asthma

severity stages, asthma control, quality of life, and exacerbations. The longitudinal part of ATLANTIS will show the relevance of the SAD score for future risks in asthma, and additionally which parameter best associates with future asthma control. Moreover, we will report on development of a Small Airways Dysfunction Tool (SADT), a questionnaire as an easy measure to suggest SAD, and on the measures of inflammation that best discriminate between the large and small airways' compartments, with bronchial and transbronchial biopsies, in a smaller subset of participants.

Funding: Chiesi Farmaceutici SpA.

Research in context

Evidence before this study

We searched PubMed for studies in asthma, including the terms asthma, adult, and small airways, and published between database inception and April 2018, using spirometry and any combination of body plethysmography, impulse oscillometry (IOS; including R5-R20 values) and Multiple Breath Nitrogen Washout (MBNW) measures, and similar terms in addition to CT scans. Small airways dysfunction (SAD) has been understudied, though it significantly contributes to airway obstruction, a hallmark of asthma. So far, studies on the role of SAD in asthma have been performed in small sample sizes and/or subgroups of asthma. Moreover, these studies investigated only a subset of available potential measurements of SAD and did not include both spirometry, body plethysmography, ~~impulse oscillometry (~~IOS~~)~~, ~~Multiple Breath Nitrogen Washout (~~MBNW~~)~~, CT scans and questionnaires.

Added value of this study

This is the largest study to date involving 773 evaluable asthma patients and 99 controls without airway obstruction specifically designed to determine the prevalence and impact of small airways dysfunction SAD in asthma. The study shows that SAD is present in asthma across all stages of severity, with highest prevalence in GINA 5. We were able to define a SAD score from a combination of lung function measurements that reflects the amount of physiological small airways impairment in asthma. The score associated significantly with measures of asthma control, history of exacerbations and disease severity. Model-based clustering delineated two clinical SAD groups that differed in age, duration of asthma, and disease severity. Of interest, values of Sacin, that measures ventilation heterogeneity in pre-acinar/acinar airways, were in the normal range in Group1. The difference between Clinical SAD Group1 and Group2 was particularly clear with clinically available SAD measurements, such as IOS and spirometry, followed by FEV_1, while differences were small with CT SAD parameters. In summary, we can cluster asthma patients in two subgroups based on SAD measured with easy-to-conduct, clinically applicable measures.

Implications of all the available evidence

Small airways dysfunction (SAD) has been understudied in asthma. Our results show the clinical relevance of SAD, which is present across all severity stages of asthma. It is particularly present in severe disease, likely reflecting structural lung changes that are not responsive to~~e~~ the use of oral corticosteroids and/or high dose inhaled corticosteroids. Moreover, SAD relates to asthma stability, severity, quality of life, exacerbation rates and health care utilization and can be delineated by easy-to-conduct, clinically applicable measures such as IOS and spirometry. Therefore, this aspect of asthma needs further consideration in the management of the disease.

Introduction

Asthma is a prevalent obstructive airway disease that affects the entire bronchial tree. The small airways, defined by a diameter ≤2 mm and referred to as the "silent zone" of the lungs, contribute to the resistance in the airways of patients with obstructive airways disease[1]. This is of clinical importance since small airways can be inflamed in asthma and hence narrowed[2-4]. Small airway narrowing can also occur due to smooth muscle contraction after inhaling allergic and non-allergic irritants. Moreover, remodeling can affect small airway wall stiffness, thereby changing their distensibility[5].

Small airways dysfunction (SAD) has been postulated to exist at all severities of asthma, whereas some studies suggest that the prevalence increases with asthma severity [6,1]. However, it is ~~still~~ not clear what proportion of asthma patients suffers from SAD, and which tests or combination of tests best defines it. Lack of best practice is due to the fact that published studies investigating the small airways in asthma included only small-sized and/or relatively homogeneous populations regarding asthma severity, or only tested one or a few physiologic SAD measures [6-8]. ~~The ATLANTIS (AssessmenT of smalL Airways involvemeNT In aSthma) study subjected a large asthma cohort to all available, clinically applicable, potential SAD tests, including spirometry, body plethysmography (e.g. residual volume), impulse oscillometry (IOS), and Multiple Breath Nitrogen Washout (MBNW) and CT scan. The physiological tests may reflect abnormalities in different parts of the bronchial tree or different aspects of small airways dysfunction, providing different perspectives on SAD[9,10]. Lung imaging by CT scan can provide additional insight regarding SAD, but the relationship with physiologic measures of SAD in asthma has not been studied extensively and only in small groups.~~

~~The ATLANTIS study assessed which (combination of) biomarkers, physiological testing and imaging markers best measures the presence and extent of SAD in asthma. It builds on both a~~

~~baseline and 1-year follow-up phase. We assessed SAD through a series of baseline measurements using published criteria defining small airways dysfunction for each test, both for physiological and CT measures. The final result of the model-building process is a score defining to what extent SAD is present in each individual patient, a score that was built from baseline data and validated at follow-up. With this score, its usefulness for prediction of asthma severity, asthma control, quality of life and history of exacerbations was evaluated~~

~~Here we present the clinical baseline data of the ATLANTIS study. The main aim is to identify which combination of biomarkers, physiologic testing and imaging approaches best measures the presence and extent of SAD in asthma cross-sectionally and their relationship with asthma severity, control, quality of life and history of exacerbations over time[9]. The study allowed us to develop novel predicted, upper limit of normal (ULN) and lower limit of normal (LLN) values of physiological parameters infrequently studied (e.g. IOS).~~ The ATLANTIS (AssessmenT of smalL Airways involvemeNT In aSthma) study is a multinational 1-year prospective cohort study, including people with asthma of all severities and controls without airway disease. In this paper we present the baseline, cross-sectional data from ATLANTIS, aiming to identify which combination of biomarkers, physiologic testing and imaging approaches best measures the presence and extent of SAD in asthma, and their relationship with features of asthma. We assess SAD through a series of all available, clinically applicable, potential SAD tests, both for physiological and CT measures. The physiological tests may reflect abnormalities in different parts of the bronchial tree or different aspects of small airways dysfunction, providing different perspectives on SAD[9,10]. Lung imaging by CT scan can provide additional insight regarding SAD, but the relationship with physiologic measures of SAD in asthma has not been studied extensively and only in small groups; here we test both physiological and CT scan measures in a large cohort.

In addition, we develop a score defining to what extent SAD is present in each individual patient and assess its usefulness for prediction of asthma severity, control, quality of life and history of

exacerbations. In future papers (not presented here), we will report the longitudinal data from ATLANTIS and aim to validate the SAD score over time, we will develop and validate a Small Airways Dysfunction Tool (SADT), a questionnaire as an easy measure to suggest SAD, and we will assess which direct and indirect measures of inflammation best discriminate between the large and small airways' compartments, with bronchial and transbronchial biopsies, in a smaller subset of participants[9].

Methods

Participants

Participants were recruited (first patient in June 30, 2014 and last patient out March 3, 2017) ~~(2014-2016)~~ from general practitioners, chest physician's databases and by advertisements in 29 centers across 9 countries worldwide. Inclusion criteria were: 1) age 18-65 years; 2) clinical asthma diagnosis ≥6 months, confirmed by a chest physician according to GINA 2012[11] and supported by objective evidence of any of the following at the baseline visit or in the previous 5 years: a) positive airway hyperresponsiveness to methacholine, ***or*** b) positive reversibility, defined as $\Delta FEV_1 \geq$ 12% and ≥ 200 mL over baseline FEV_1 within 30 minutes after inhaling 400 µg of salbutamol pMDI with or without a spacer ***or c)*** Peak Expiratory Flow variability (i.e. highest - lowest value over the day/mean value of the two, ×100) >20%, measured during 7 days ***or*** d) documented reversibility after a cycle (e.g. 4 weeks) of maintenance anti-asthma treatment; 3) stable asthma on any previous regular asthma treatment ("rescue" β2-agonists alone included) at a stable dose for ≥ 8 weeks before baseline; 4) lifetime smoking ≤ 10 pack-years. Main exclusion criteria were a COPD diagnosis confirmed by a chest physician and an asthma exacerbation during 8 weeks before baseline.

Controls were included based on 1) age 18-65 years; 2) no respiratory symptoms compatible with asthma or COPD in the past 2 years; 3) normal spirometry: baseline $FEV_1 \geq$ 80%predicted, FEV_1/Forced Vital Capacity (FVC)> LLN (lower limit of normal); 4) normal airways responsiveness: PC20≥ 16 mg/mL, PD20≥ 1.4 mg; 5) lifetime smoking ≤ 10 pack-years. Diagnosed upper/lower respiratory tract diseases were exclusion criteria. The Medical Ethics Committee of each center approved the protocol; all patients gave written informed consent.

Study design and procedures

Participants were followed for 1 year with 6-month clinic and 3-month telephone follow-ups[9]. The clinical and CT tests were performed at 3-day baseline visits. The methods for spirometry,

hyperresponsiveness, MBNW, IOS, body plethysmography, CT, questionnaires, blood tests, and health care utilization are described in the Supplement. Medications during an eight-week period before evaluation were used to assess GINA severity[11]. The potential indices of SAD used with hypothetical location in the airways and references between brackets are presented in Table 1 [12-19~~22~~] . These were % fall in FVC during hyperresponsiveness testing; spirometry: Forced Expiratory Flow $(FEF)_{25-75}$, ~~and~~ FEF_{50}, both corrected for FVC; ~~,~~ body plethysmography: Residual Volume/Total Lung Capacity (RV/TLC), ~~and~~ Functional Residual Capacity (FRC), IOS: R5-R20, AX, ~~and~~ X5; ~~-~~MBNW: Scond, ~~and~~ Sacin. Alveolar NO was not incorporated in this analysis since it was only available in a subset of participants (~~See~~ Supplement). Indices of "large airways dysfunction", which may also capture small airways abnormalities, were FEV_1%predicted, FEV_1/FVC, IVC, FeNO, R20, PC_{20}, PD_{20} and 3 severity categories of airway hyperresponsiveness (Supplement).

Computed tomography

Volumetric whole lung scans were obtained at full inspiration (near total lung capacity) and at end of expiration, near FRC. Scans were analyzed by a single observer (SB) using semi-automated software, Apollo (VIDA Diagnostics, Iowa), with various quality control parameters[20~~3~~,21~~4~~]. The supplement describes CT acquisition, quantitative airway morphometry and lung densitometry. SAD parameters used (~~see also~~ Table 1 [21~~4~~,22~~5~~]) were: ex- and inspiratory Mean Lung Density and their ratio (E/I MLD), ex- and inspiratory lung volume and their ratio (E/I LV), expiratory Voxel Index (VI-856) and inspiratory VI-950 (% of Voxels with CT numbers <-856 and <-950 Hounsfield Units respectively, inspiratory Percentile15, Inspiratory median Lumen area, Wall area (WA) and Total area, these latter three divided by body surface area (BSA), inspiratory median percentage WA, and inspiratory Pi10 and Po20%WA (hypothetical airway with internal perimeter of 10 mm and outer perimeter of 20 mm respectively).

Statistical analyses

Detailed statistical information, including power analysis[263], is provided in the Supplement. The following variables reflecting SAD were used in the clinical SAD analysis: FEF_{50}/FVC, FEF_{25-75}/FVC, FEV_1%predicted, FEV_1/FVC, IVC%predicted, % fall FVC at PC_{20} or PD_{20}, RV/TLC %predicted, FRC%predicted, R5-R20, X5, AX, Scond, Sacin. For CT SAD analysis, variables were: MLD ratio, Lung Volume ratio, VI-856, Pi10, Po20%WA.

We used SEM analysis to assess clinical SAD, since this clarifies which SAD parameters, out of all the physiologic parameters ~~we~~ measured~~.~~, group together and weigh towards the presence of SAD in asthma (~~see~~ Supplement). Similarly we applied SEM analysis for CT SAD. Several steps were performed for clinical SAD and CT SAD SEM analysis separately[274]. A correlation matrix evaluated correlations among observed variables, high correlations indicating presence of underlying latent variables. An exploratory factor analysis for observed variables was performed to identify the underlying SAD factor structure. The final underlying SAD factor structure was tested by specifying a confirmatory factor model. Once the measurement model was set and fit the data properly, it was used to classify each patient into SAD groups, using model-based clustering. The SAD Groups and SAD scores from the clinical SAD and the CT-scan SAD model were compared, evaluating the rate of agreement, using Chi-square and Pearson's correlation tests. The clinical SAD model was additionally tested in the subgroup with a CT scan, by adding the CT scan variables to the model. Full information maximum likelihood (FIML) method was used for dealing with missing data in SEM analysis[258].

Relationships of physiologic SAD variables with asthma severity, control and healthcare utilization were analyzed by Poisson regression. Continuous prediction equations, their lower- and upper limit of normal (LLN and ULN) from the literature[296] and from formulas based on ATLANTIS controls are provided in Supplemental Table 1. Statistical analyses and data processing were performed

using Statistical Analysis Systems (SAS®) Software (release 9.2) and Mplus Version 7.4 on a Windows 7 operating system.

Role of Funding Source

Chiesi Farmaceutici SpA financed the study, contributed to the set-up of the study which was designed by DP, MK, CB, MvdB, LF, AP, TvdM, KR, SS and DS. Chiesi Farmaceutici SpA contributed to interpretation of the study and approved the submitted manuscript. Data collection and management was done by Cromsource and data were analysed by CROS NT. All co-authors discussed and interpreted the data. The first draft of the report was written by DP, CB and MK; DP collated input from all co-authors. DP and MK had access to raw data. The corresponding author had full access to all of the data and the final responsibility to submit the initial and revised manuscript.

Results

The main reason for screening failure was not fulfilling inclusion/exclusion criteria (n=99, Figure 1).

Participants

Baseline characteristics are shown in Table 2, Table 3 (asthma only) and Supplemental Table 2. Gender, age and smoking habits were comparable between asthma and control participants; the large majority of people included were of Caucasian descent (88% and 96% in asthma and control participants respectively). Asthma participants demonstrated higher BMI, heart rate, blood pressure, blood cell counts, and prevalence of atopy. Hyperresponsiveness was only present in asthma participants. All physiologic parameters were significantly worse in asthma. Asthma participants had lower MLD expiratory values, inspiratory airway lumen, wall, and total area, also when divided by BSA ~~(body surface area)~~ on CT. Asthma participants had a moderately severe health status impairment (Table 3) and lower lung-related quality of life (higher EuroQol-5Dim-5Levels score) than controls, median (Q1;Q3) ~~value of~~being 95.0 (90.0;100.0) versus 80.0 (70.0;90.0).

Association of physiologic parameters with asthma severity, control and health care utilization

X5, Scond, RV/TLC, R5-R20 and R5 values (Figure 2A) showed the highest positive correlations with GINA severity[11]. GINA severity was also associated, as expected, with lower FEV_1, FEF_{50}, and FEV_1/FVC values. Table 4 shows that GINA5 had the highest SAD prevalence rate for every physiologic variable (measurements >ULN or <LLN). Sacin had the least SAD prevalence rate in all GINA stages, the lowest prevalence being with GINA1 (12%), rather similar, higher prevalences in GINA2-4 (18-19-20%), and highest in GINA5 (41%). This contrasts with other SAD variables, where prevalences either remain constant over the GINA stages (% fall FVC), continuously increase from GINA1-GINA5 (body plethysmography), or increase in steps, e.g. Scond and FEF_{25-}

$_{75}$ showed lowest prevalences in GINA1-2, higher in GINA3-4 and highest in GINA5. R5-R20 and AX showed somewhat comparable rates in GINA1-3, higher in GINA4 and highest in GINA5 (Table 4). Sacin also contrasted with <LLN prevalence distributions in FEV_1, i.e. GINA1-GINA5 26%-29%-36%-47%-72%.

A lower Asthma Control Test (ACT) score was particularly associated with higher AX and R5 and lower FVC and FEV_1 (Figure 2B).

For exacerbations in the past year, highest positive correlations were with RV/TLC, R5-R20, AX and Sacin and highest negative correlations with FEV_1, FVC, IVC, FEF_{25-75}, FEF_{50} (Figure 2C). The number of exacerbations was independently predicted by SAD parameters from spirometry, IOS, body plethysmography, hyperresponsiveness severity, female gender and height (Table 5). There was also a negative association with Raw. Independent parameters for unscheduled consultation visits were FEV_1, hyperinflation with body plethysmography, hyperresponsiveness severity, and female gender (Table 5).

Prevalence of LAD and SAD in asthma

Figure 3 (upper panel) shows the prevalence rates of large and small airways dysfunction, based on LLN and ULN. Sacin had the lowest SAD prevalence (19.2%), % fall FVC the highest (73.1%).

SAD Model

Figure 4 shows the final clinical SAD model based on cross-sectional data. It presents both the loadings to the three latent variables, and the goodness of fit values (Supplemental methods), showing good coherence of this model to SAD. IOS parameters R5-R20, AX and X5 loaded to the first latent variable, FEF_{50} and FEF_{25-75} both corrected for FVC, to the second latent variable, while Sacin (MBNW) loaded both to the first and second latent variable. The lung volume parameter RV/TLC %predicted and Scond (MBNW) loaded to the third latent variable. Hyperresponsiveness was only tested at the first visit, hence could not be taken into account in the longitudinal design of

the SAD SEM model. Therefore, we also analyzed the clinical SAD model at baseline including hyperresponsiveness, and the % fall FVC loaded on the third latent variable without much change in goodness of fit values. The baseline model without and with % fall FVC correlated highly (r=0.99; Supplemental Figure 2A). Since the cross-sectional SAD model with and without % fall FVC were almost identical, the model without % fall FVC was tested longitudinally; the same model structure was confirmed at all visits (Supplemental Figure 2B).

Correlations of clinical SAD score with physiologic and clinical parameters

A higher clinical SAD score reflects more severe SAD. The highest positive and negative correlations ($r > 0.60$ and $r < -0.60$) of the SAD score existed with physiologic parameters on which the score was based, i.e. IOS parameters AX, R5-R20, and R5 (positively) and X5, spirometric parameters FEF_{25-75} and FEF_{50} (negatively), next being FEV_1 %predicted (Figure 5). The highest correlations of non-physiological parameters with the SAD score were duration of asthma, ACQ-6 and number of exacerbations (positively), ACT, Mini AQLQ total and EQ-5D-5L (negatively). Clinical SAD scores increased with higher asthma severity, mean SAD score in GINA1-5 being -0.143, -0.035, -0.048, +0.071 and +0.239 (ANOVA $p < 0.0001$).

Model-based clustering defined clinical SAD Groups

Model-based clustering defined two clinical SAD groups, Group1 including 452 patients, Group2 312 patients (Table 6 and Supplemental Table 3 present clinical characteristics). Overall, the 2 clinical SAD Groups were similar regarding age of asthma onset, sex ratio, FeNO, atopy, and smoking habits, while duration of smoking was higher in Group2 (Table 6). Sacin values were comparable between Group1 and the controls, whereas Group2 had significantly higher values than both Group1 and controls. Clinical SAD Group2 was somewhat older, demonstrated higher blood pressure, heart rate and BMI, and a longer asthma duration. Additionally, Group2 had more severe asthma than Group1, according to GINA severity, ACT, ACQ, LABA/ICS use,

hyperresponsiveness, blood inflammation (eosinophils), quality of life and health care utilization. All physiologic parameters were worse in clinical SAD Group2; the two groups were best separated by SAD parameters from IOS followed by spirometry, and additionally FEV_1 (Figure 3).

CT scan factors in SAD

CT scans were analyzed in 294 patients (with comparable asthma severity as the non-CT group, Supplemental Table 3). The SEM model provided three factors in CT that contributed to SAD: MLD inspiratory/expiratory ratio, Lung volume inspiratory/expiratory ratio and VI-856 (Supplemental Figure 2D). The correlations of the CT SAD score with physiologic and clinical parameters, comparison of CT SAD groups, and additional Clinical SAD analysis in patients who had a CT scan are presented in the Supplement.

Relationship between Clinical and CT SAD scores

The Clinical SAD and CT SAD scores showed a significant, weak correlation (r=0.28). There was no significant overlap between the clinical SAD and CT SAD Groups (p=0.103, Supplemental Table 5).

Discussion

This large ~~clinical~~ study shows the clinical relevance of small airway dysfunction for asthma, since SAD is present across all severities and particularly in more severe asthma. ATLANTIS was specifically designed to determine the prevalence and impact of SAD in asthma and has performed the most comprehensive evaluation of SAD to date using both physiological and imaging tools. We show that the prevalence of SAD depends on the physiologic measure used, i.e. localization and type of airway narrowing. Of importance, no single variable defines SAD, but IOS, MBNW, lung volumes and spirometry all contribute. For clinical practice, it is important to highlight that SAD associates with GINA severity and ~~-~~ independently- with history of exacerbations over time, particularly when measured by IOS, spirometry and body plethysmography. Moreover, the poorest asthma control was present in the group with the worst clinical SAD score.

Of note, 91% of our asthma population expressed SAD, ~~when~~ defined as any abnormal physiologic parameter. This does not imply that patients ~~Our data imply that they do~~ have extensive SAD throughout all airway dimensions, since the prevalence varied with the type of physiologic measure. The lowest prevalence existed with Sacin (19%) and RV/TLC (22%), both reflecting dysfunction of the most peripheral small airways. The highest prevalence was with FEF_{25-75} (68%) and % fall FVC (73%), probably both reflecting obstruction in more small-to-mid-sized airways. Future work has to elucidate if these different prevalence rates define subtypes of SAD ~~(consistent vs. variable, which level of airway is involved, and what percent of these airways are involved).~~ We additionally compared our SAD prevalence with literature findings (Supplemental Table 7), yet no study compared all types of physiologic SAD methods. Anderson et al.[6] used R5-R20 >0.03 kPa/L/s as cut-off for abnormality, concluding that abnormal R5-R20 values were present in all severities of asthma, i.e. 65% in British Thoracic Society step2, 64% in step3 and 70% in step4. Our overall prevalence with this cut-off was 70%; we extend their findings showing that prevalence rates of R5-R20 >LLN increase from GINA steps 1-5, being 54%, 65%, 70%, 77%, and 91% respectively. In

contrast, the prevalence of Sacin >LLN was lowest in GINA1, almost identical in GINA 2-4 and highest in GINA5, suggesting that mostly peripheral airway dysfunction, and likely structural changes, are present in most severe asthma. In summary, our data are comparable with published findings in smaller samples, yet expand these observations by providing information on all different SAD measurements at the same time in one group of asthma patients across all severities.

Strengths of our study are the large group of asthma patients covering the full severity spectrum and the extensive work-up and quality and experience of the centers. ATLANTIS is a multi-center international study, therefore we feel our results are reliable and applicable to multiple populations. We also included smokers, a factor that by itself may induce some SAD. We felt it important that our study reflects the larger asthma and non-asthma population globally for generalizability, and thus not restricts the impact of our findings. The controls had comparable age, sex ratio and particularly smoking habits as the asthma population, which provided novel LLN and ULN values for physiological parameters infrequently studied, likesuch as IOS and MBNW. We acknowledge that a larger control group might have improved precision of these predicted, LLN and ULN values, whichand this will be also partially overcome when we add the longitudinal data in the future.

We recognize that a quality check of the maneuver to get optimal phase III slope in the MBNW test[3027] is key to validity of the measurements, which we have carefully ensured in the present study. The finding that of some measurements of ventilation heterogeneity in pre-acinar/acinar airways (Sacin values were) in the normal range doesis not in contrast with the presence of airway dysfunction in Group1, as the body of the available literature on ventilation heterogeneity in adult asthma[214,2831-384] reveals a variable contribution of conducting versus acinar lung regions to treatment response, and consistency in the reversibility towards normal values after exacerbations[3281]. Particularly, the persistent derangement of ventilation in conducting airways (Scond) seems more related to airway remodeling, exacerbations, and hyperresponsiveness, whereas the reversible derangement in acinar airway ventilation (Sacin) mainly reflects asthma

severity[3~~9~~5]. Accordingly, the worst clinical SAD score was present in the group with the poorest asthma control and higher prevalence in GINA 4 and 5.

Another limitation ~~of the study~~ is that CT scans were not available in all participants, limiting numbers for analyses. However, this allowed us to demonstrate that the clinical SAD model in the full asthma cohort could be replicated in the smaller group with CTs. Future work will expand our analyses by performing parametric response mapping (PRM)[4~~0~~36], a CT voxel-based imaging biomarker tool ~~which uses dynamic image registration between paired inspiratory and expiratory scans~~ to quantify 'functional small airways disease'. A potential limitation is that age~~there was a~~ was somewhat higher ~~age~~ in the asthma than control participants, yet the difference ~~is was a~~ small ~~difference~~ (median age (interquartile ranges) of 46 (34-54) vs 41 (29-52) years respectively) and~~that is~~ likely not of clinical significance, and we adjusted for age in all analyses. We cannot put our clinical SAD score forward as a clinically applicable tool as yet, since this is a cross-sectional analysis~~study~~. The score already significantly associates with number of exacerbations, asthma severity and control, and the longitudinal phase of the study will elucidate whether it also predicts future changes in these clinical outcomes. For the same reason we cannot put the "best parameters" of SAD forward yet~~, since this also needs prospective data that will follow in the future~~. Additionally, a Small Airways Dysfunction Tool (SADT) will be developed, a questionnaire as an easy measure to suggest SAD[9], which may be easily applicable in the clinic~~,~~ as MBNW and body plethysmography are not available for all routine settings. Our article did not report on SAD with regard to the underlying pathology[9]. ~~It was not feasible to perform bronchial and transbronchial biopsies in all participants.~~ However, we will assess which direct and indirect measures of inflammation best discriminate between ~~the~~ large and small airways' compartments, with bronchial and transbronchial biopsies in a smaller subset of participants in the future.

Large and small airways obstruction are important components of asthma pathophysiology[1-3]. Our focus ~~in this study~~ is on the small airways and their specific impact upon asthma symptoms and exacerbations, an area of investigation that has been relatively neglected in our opinion (an overview of relatively small-sized studies is presented in Supplemental Table 7). It would be of interest to analyze in the future ~~data in individuals~~subgroups with Large Airway Dysfunction (LAD) without SAD, or conversely, ~~individuals~~ with SAD and without LAD. Finally, one would like to have a 'gold standard' for SAD, yet our study shows this is not feasible since many physiological parameters contribute to the SAD model. This likely reflects that they represent abnormalities in distinct parts of the bronchial tree and/or contrasting aspects of underlying mechanisms of SAD, thereby providing different information[9].

We were able to define a SAD score that reflects the amount of physiological small airways impairment and is significantly associated with ~~measures of~~ asthma control, exacerbations and severity. We additionally observed two clinical SAD Groups that are comparable in e.g. gender, atopy, FeNO, ICS dose and smoking habits, while Group2 was somewhat older, had a longer asthma duration and more severe asthma according to all parameters tested. Interestingly~~Of interest~~, ~~ventilation heterogeneity in pre-acinar/acinar airways measured as~~ Sacin)[32], which reflect~~sive of~~ dysfunction of the most peripheral small airways, was in the normal range in Group1 only and had a higher prevalence in Group2. The difference between the two clinical SAD g~~G~~roups~~1 and Group2~~ was particularly clear with SAD measurements like IOS and spirometry (Figure 3). Clinical SAD Group 2 represents "more severe" SAD, given particularly the presence of more severe small-to-mid-sized airway obstruction (R5-R20, FEF_{25-75}) and less airway distensibility (AX). In summary, we can detect asthma subtypes based on presence and extent of SAD measured with easy-to-conduct, clinically applicable tools.

Similarly, with regard to the clinical SAD score, we developed a CT-SAD score. The CT-SAD score significantly associated with GINA severity, but less well than the clinical SAD score. CT

SAD Group2 had more severe asthma and the physiologic parameters were significantly different from controls and from Group1. However, the CT SAD Groups had similar levels of small-to-mid-sized airway obstruction (R5-R20) and conducting airway ventilation heterogeneity (Scond), reflective of dysfunction in small-medium size conducting airways, while Group2 had significantly higher air trapping (RV/TLC) and acinar airway ventilation heterogeneity (Sacin) values, reflective of the most peripheral small airways. This suggests that CT scan-derived SAD captures regional differences in mechanisms of airway dysfunction due to air trapping ~~and small airways~~ as a surrogate for peripheral airways impairment[41]. They become apparent in supine position, when airway closure and compliance reduction develop as consequence of severe hyperinflation and expiratory reserve volume reduction[42][37] in ~~participants with~~ more severe asthma. Notably, we observed a difference in airway distensibility (AX) in participants undergoing CT scan, in comparison to those who did not (~~see~~ Supplemental Table 3). It is thus understandable that the Clinical SAD score and the CT SAD score were not concordant (r=0.28). Where CT scans (performed in supine position) provide information on SAD particularly by changes driven from increased residual static lung volumes and air trapping[43][38], the physiologic parameters measured in the sitting position provide information on air trapping (body plethysmography RV/TLC), small airway obstruction (IOS and FEF_{25-75}) and heterogeneity of both conducting and acinar airway ventilation (MBNW). This potentially explains why the CT SAD score, in contrast to the clinical SAD score, did not associate with health status or asthma control.

Asthma control is lacking in 50-60% of patients despite guideline-based management[44][39] and untreated SAD has been proposed as a contributing factor[1]. Drivers of asthma control include treatment adherence and appropriate use of inhalers, psychological factors and environmental trigger exposures. The current study suggests that asthma control is also determined by the presence of SAD, since ACT was significantly associated with the clinical SAD score and ~~was~~ specifically

abnormal in clinical SAD Group2 (most severe SAD). Moreover, a lower ACT score was associated with higher IOS parameters R5 and AX values. ~~These data suggest that~~Hence asthma control may be partially driven by SAD, but also obstruction in larger airways given its association with FEV_1, the gold standard for diagnosis and severity in clinical practice.

~~Of note, 91% of our asthma population expressed SAD when defined as any abnormal physiologic parameter. Our data imply that they do not all have extensive SAD throughout all airway dimensions, since the prevalence varied with the type of physiologic measure. The lowest prevalence existed with Sacin (19%) and RV/TLC (22%), both reflecting dysfunction of the most peripheral small airways[41]. The highest prevalence was with FEF_{25-75} (68%) and % fall FVC (73%), probably both reflecting obstruction in more small-to-mid-sized airways. Future work will have to elucidate if these different prevalence rates define subtypes of SAD (consistent vs. variable, which level of airway is involved, and what percent of these airways are involved). We additionally compared our SAD prevalence with literature findings (Supplemental Table 7), yet no study compared all types of physiologic SAD methods. Anderson et al.[6] used R5-R20 >0.03 kPa/L/s as cut-off for abnormality, concluding that abnormal R5-R20 values were present in all severities of asthma, i.e. 65% in British Thoracic Society step2, 64% in step3 and 70% in step4. Our overall prevalence with this cut-off was 70%, while our data extend their findings showing that the prevalence rates of R5-R20 >LLN increase from GINA steps 1-5, being 54%, 65%, 70%, 77%, and 91% respectively. In contrast, the prevalence of Sacin >LLN was lowest in GINA1, almost identical in GINA 2-4 and highest in GINA5, suggesting that mostly peripheral airway dysfunction, and likely structural changes are present in most severe asthma. In summary, our data are comparable with published findings in smaller samples, yet extend these observations by providing information on all different SAD measurements at the same time in one group of asthma patients across all severities.~~

Of interest, asthma participants had higher blood pressure than our controls. We did not find literature reporting this observation. Comorbidities are thus not only present in COPD, another obstructive pulmonary disease [45.46]40,41, but also occur in asthma patients with a median~~n average~~ age of 46 (34;54) years. This~~e finding~~is is in agreement with previous studies indicating systemic inflammation as one underlying mechanism linking reduced lung function to cardiovascular mortality[47]42 and a positive association between lower FEV_1 and systemic arterial hypertension, while lower ICS doses attenuated the likelihood for hypertension in a population with comparable~~of the same~~ age to~~as~~ ours[43]38. Alternatively, hyperinflation could ~~be also considered to~~ have a role via its contribution to changes in intrathoracic pressure that increase left ventricular wall stress, similar to ~~what has been~~ report~~sed~~ in COPD[44]49.

In conclusion, our data in a large asthma population covering the full spectrum of asthma severity show the complexity of SAD. Notwithstanding this, the clinical classification of Small Airways Dysfunction is meaningful given its association with asthma severity, control and exacerbations. Results show that SAD can be present across all GINA severity stages. Depending on the type of physiologic parameter used, the prevalence rate changes considerably, but is consistently ~~the~~ highest in GINA5. SAD prevalence rates were lowest with Sacin, reflecting pre-acinar/acinar airway abnormalities, and this prevalence was quite comparable over GINA2-4 but again highest in GINA5, suggesting structural abnormalities in severe asthma. In contrast, other physiologic parameters showed either increasing prevalence rates with severity (RV/TLC) or a stepwise increase (FEF_{25-75}, R5-R20, AX, X5). Clinical SAD and CT SAD scores did not significantly correlate. SAD derived from the CT scan provides particularly data on air trapping and ventilation impairment in more peripheral airways, while the physiologic measures show results from both small-medium size conducting airways ~~-~~and peripheral airways. For clinical practice it is important

that physiological, easy-to-conduct measures ~~such as~~like IOS and spirometry, delineate two asthma SAD subtypes that differ in exacerbation rates, quality of life, asthma severity and control.

Contributors

DP, MK, CB, MvdB, LF, AP, TvdM, KR, SS and DS designed the study. DP, MK, CB, MvdB, LF, GA, AP, TvdM, KR, SS, NG and DS discussed and interpreted the data. The first draft of the paper was written by DP, CB and MK; DP collated input from all co-authors who reviewed all versions of the manuscript. DP and MK had access to raw data. The corresponding author had full access to all of the data and the final responsibility to submit the initial and revised manuscript.

Declaration of interests

D.P. reports that the University of Groningen has received money regarding a research grant from Astra Zeneca, Chiesi, Genentec, GSK and Roche, regarding consultancies from Astra Zeneca, Chiesi, and GSK, outside the submitted work. CB reports grants and personal fees from Chiesi, grants from AirPROM, during the conduct of the study; grants and personal fees from GlaxoSmithKline, AstraZeneca/Medimmune, Boehringer Ingelheim, Novartis, Chiesi, Roche/Genentech, personal fees from Vectura, Theravance, PreP, Gilead, Sanofi/Regeneron Teva, grants from Pfizer and Mologic, personal fees from Gossamer and 4DPharma, outside the submitted work. SB reports personal fees from Chiesi SAS FRANCE, during the conduct of the study; personal fees from employment, outside the submitted work. MvdB reports research grants paid to University from Chiesi, Teva Pharma, GlaxoSmithKline, outside the submitted work. LF reports personal fees, non-financial support and other from Chiesi Farmaceutici, during the conduct of the study; grants, personal fees and non-financial support from Boehringer Ingelheim, Chiesi Farmaceutici, GlaxoSmithKline, Merck Sharp & Dohme, Takeda, AstraZeneca, Novartis,Menarini; personal fees and non-financial support from Pearl Therapeutics and Mundipharma ,personal fees from Zambon, outside the submitted work. AG and GN report employment by Chiesi Farmaceutici

S.p.A. which sponsored the study. AP reports grants and personal fees from Chiesi Pharmaceuticals, during the conduct of the study; grants, personal fees, non-financial support and other from Chiesi, Astrazeneca, GlaxoSmithKline, Boehringer Ingelheim, Mundipharma and personal fees and non-financial support from Menarini, Pfizer, Novartis, Zambon, outside the submitted work. TvdM reports grants and personal fees from Astra Zeneca, TEVA, GSK, personal fees from Boehringer Ingelheim, outside the submitted work; and From 1 June 2017 T van der Molen is a part-time employee of GSK. KR reports personal fees from AstraZeneca, Boehringer Ingelheim, Novartis, Sanofi, Teva, Intermune, Chiesi Pharmaceuticals, Berlin Chemie and grants from Ministry of Education and Science, Germany outside the submitted work. SS reports grants from Chiesi onulus foundation, Sir Jule Thorne Trust, Medical Research Council/EPSRC NAPP, NIHR UK, and personal fees from AZ, GSK, Boehringer Ingelheim, Novartis, Mundipharma, Owlstone, outside the submitted work. D Singh reports grants and personal fees from Chiesi, AstraZeneca, Boehringer Ingleheim, GlaxoSmithKline, Glenmark, Merck, Menarini, Mundipharma, Novartis, Peptinnovate, Pfizer , Pulmatrix, and personal fees from Cipla, Apellis, Genentech, Skyepharma, Teva, Therevance, and Verona, outside the submitted work. MK reports grants from NIH, Chiesi, Sanofi, personal fees from Elsevier, during the conduct of the study.

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Figures and Tables

Figure 1. Flow-diagram of patients and controls without airway obstruction, with reasons for drop out

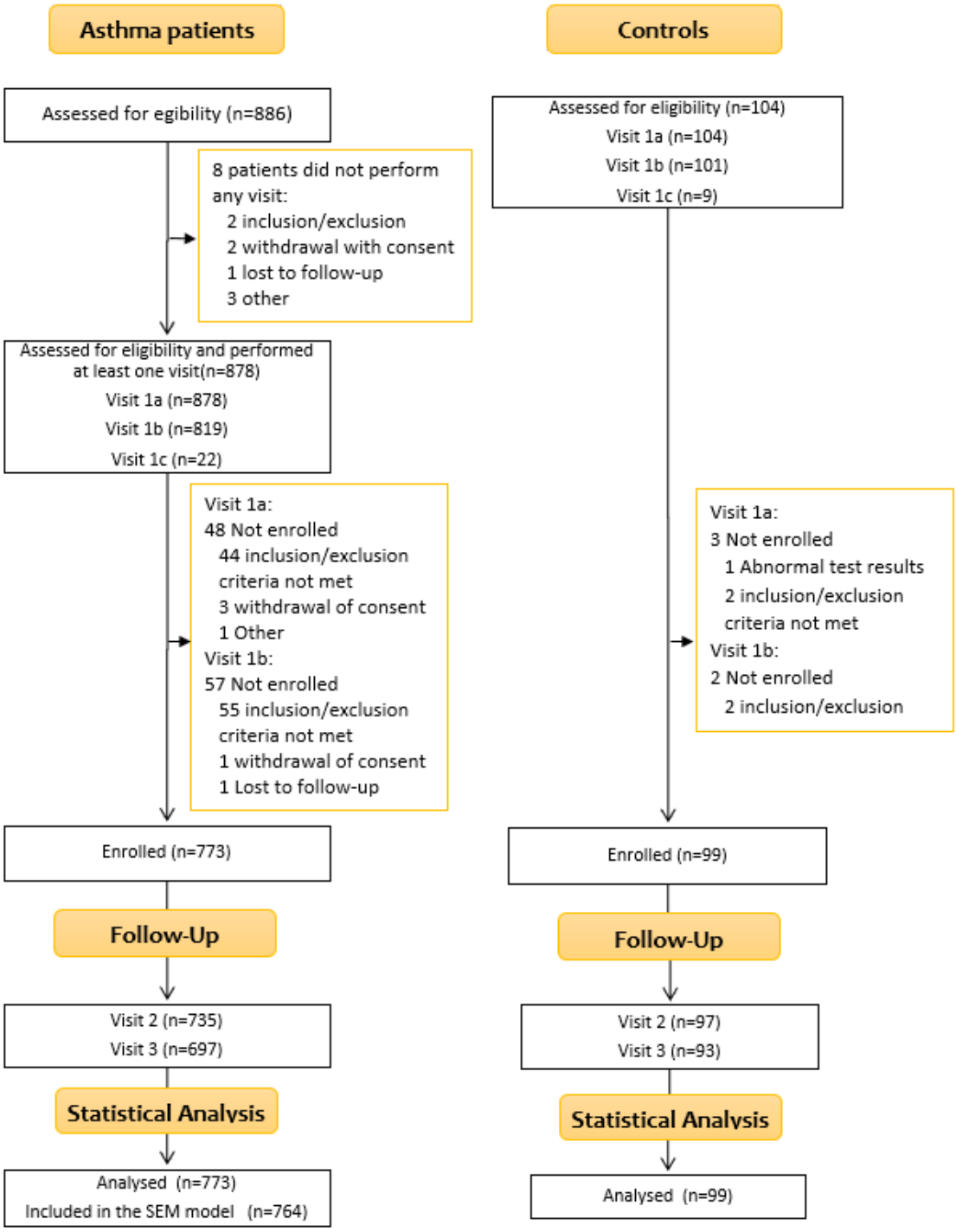
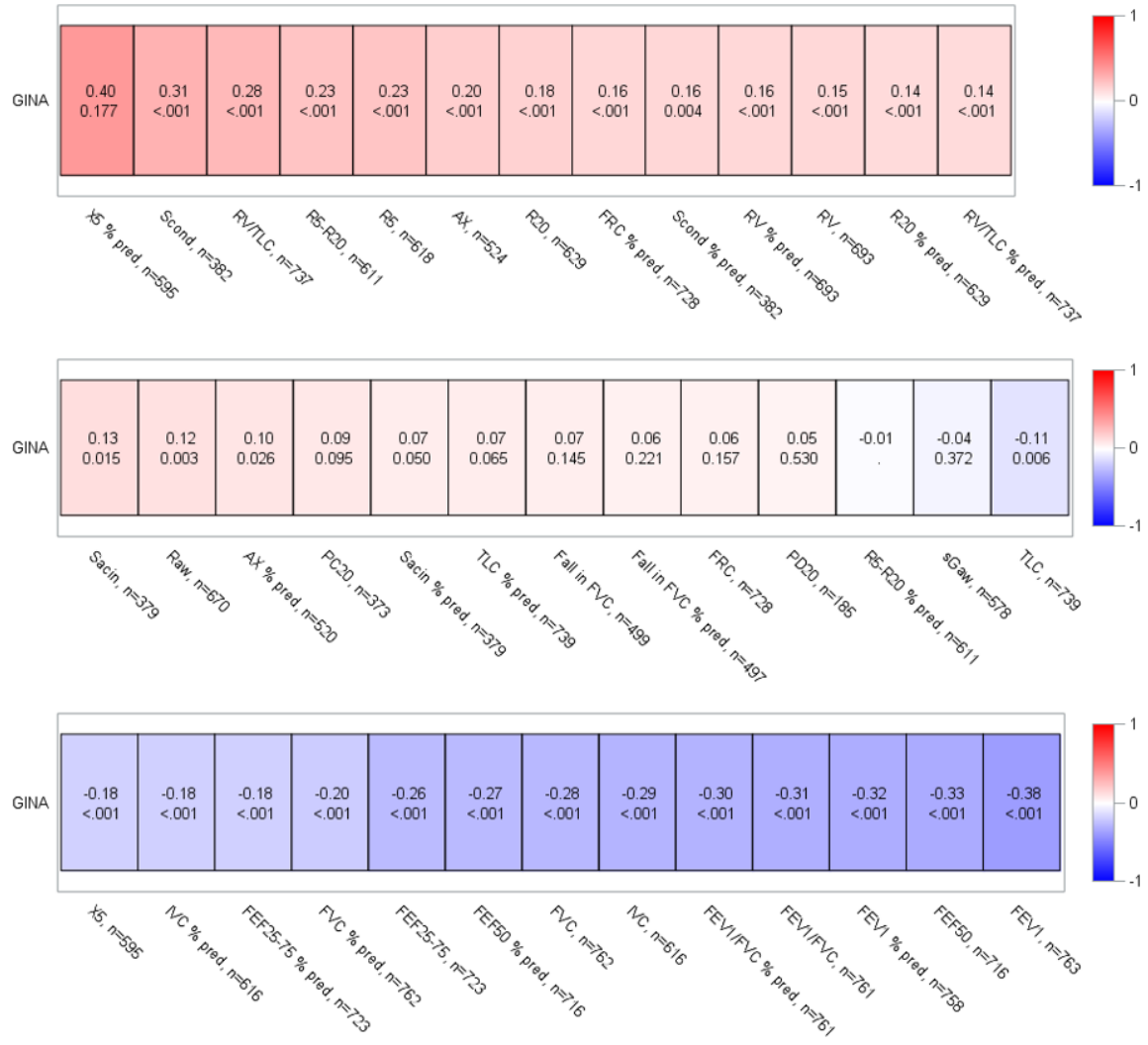
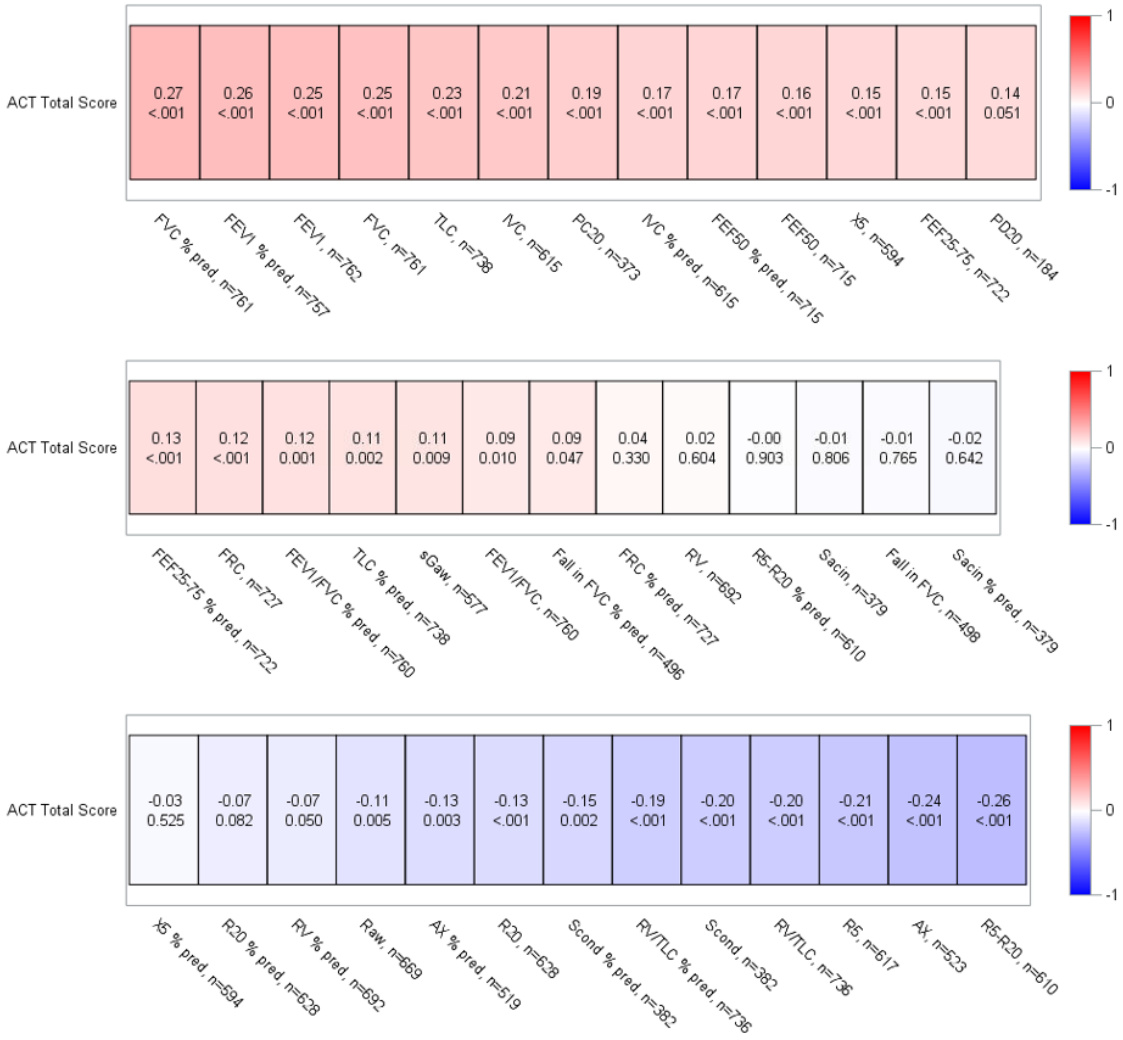


Figure 2. Monovariate correlations of physiological parameters and GINA severity, ACT score and number of exacerbations




ACT Total Score
0.27 <.001
0.26 <.001
0.25 <.001
0.25 <.001
0.23 <.001
0.21 <.001
0.19 <.001
0.17 <.001
0.17 <.001
0.16 <.001
0.15 <.001
0.15 <.001
0.14 0.051
FVC % pred, n=761
FEV1 % pred, n=757
FEV1, n=762
FVC, n=761
TLC, n=738
IVC, n=615
PiC20, n=373
IVC % pred, n=615
FEF50 % pred, n=715
FEF50, n=715
X5, n=594
FEF25-75, n=722
PiD20, n=184
1
0
-1
ACT Total Score
0.13 <.001
0.12 <.001
0.12 0.001
0.11 0.002
0.11 0.009
0.09 0.010
0.09 0.047
0.04 0.330
0.02 0.604
-0.00 0.903
-0.01 0.806
-0.01 0.765
-0.02 0.642
FEF25-75 % pred, n=722
FRC, n=727
FEV1/FVC % pred, n=760
TLC % pred, n=738
sGaw, n=577
FEV1/FVC, n=760
Fall in FVC % pred, n=496
FRC % pred, n=727
RV, n=692
R5-R20 % pred, n=610
Sacin, n=379
Fall in FVC, n=498
Sacin % pred, n=379
1
0
-1
ACT Total Score
-0.03 0.525
-0.07 0.082
-0.07 0.050
-0.11 0.005
-0.13 0.003
-0.13 <.001
-0.15 0.002
-0.19 <.001
-0.20 <.001
-0.20 <.001
-0.21 <.001
-0.24 <.001
-0.26 <.001
X5 % pred, n=594
R20 % pred, n=628
RV % pred, n=692
Raw, n=669
AX % pred, n=519
R20, n=628
Scond % pred, n=382
FV/TLC % pred, n=736
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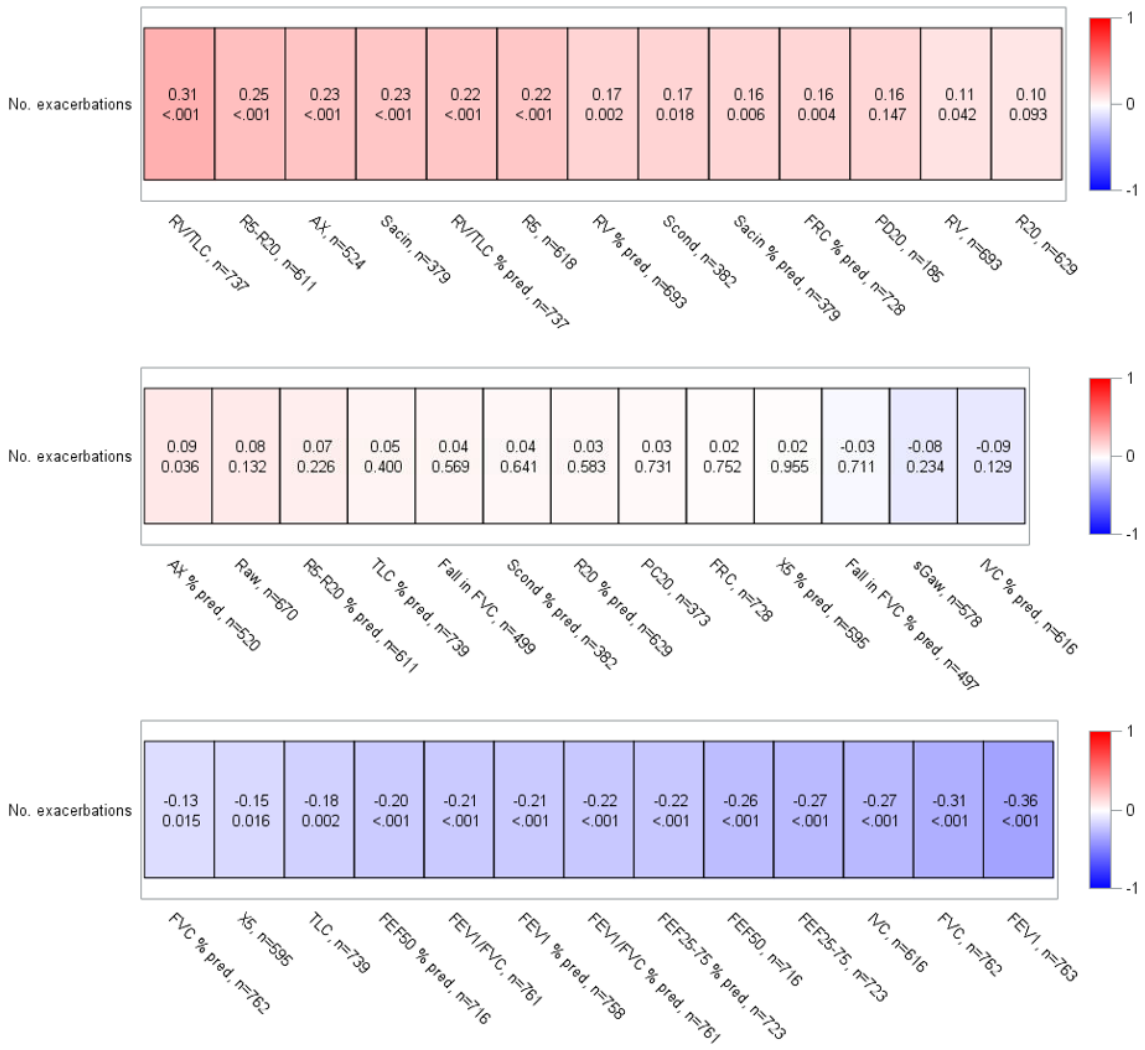
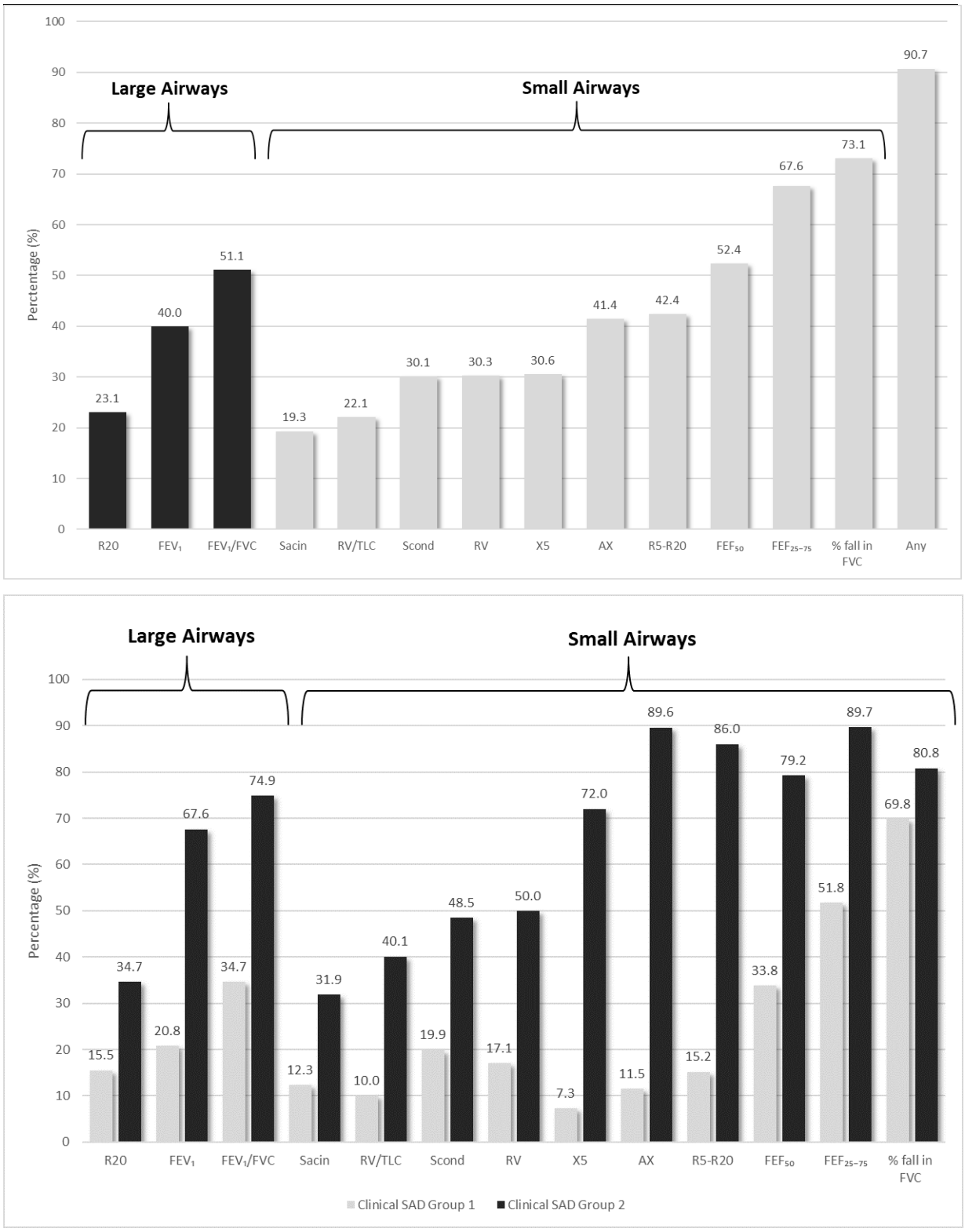
R5-R20, n=610
1
0
-1



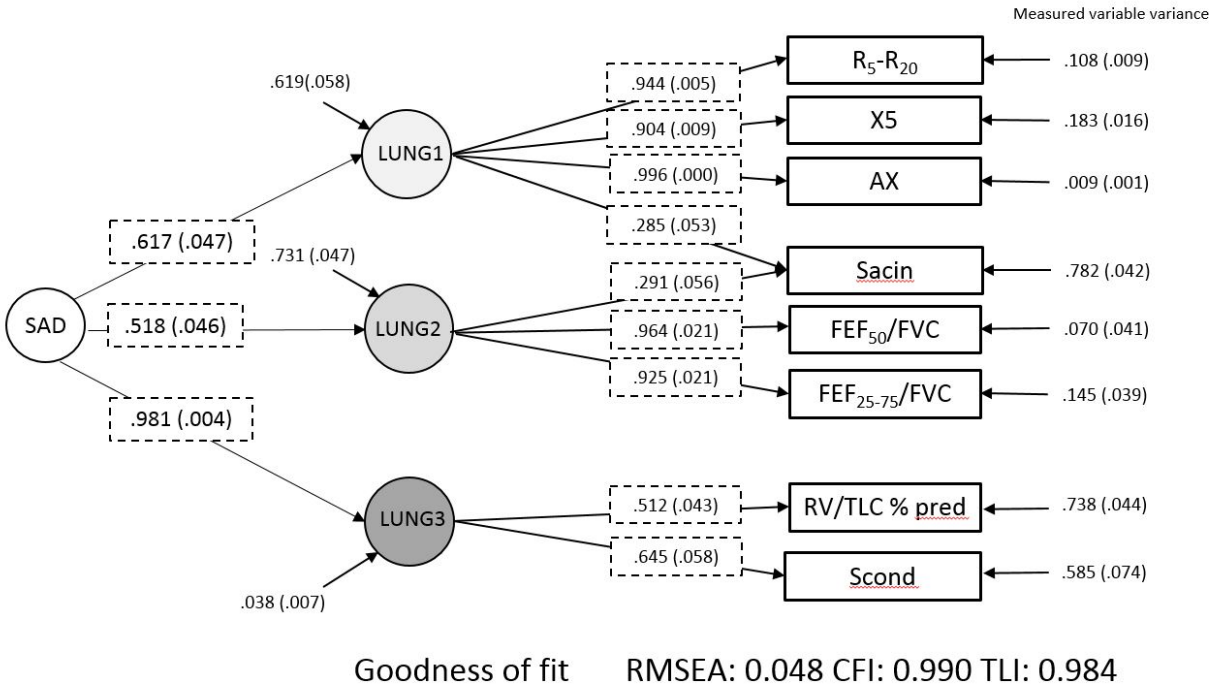
Legend to Figure 2. Correlations are presented for GINA severity (top panel), ACT score (middle panel), and Number of exacerbations in the past year (lowest panel). Darkest red is highest positive correlation between parameters. Darkest blue is the lowest negative correlation between parameters. All abbreviations are presented in Table 1.

Figure 3. Prevalence rates of airways dysfunction in the full asthma cohort and in the 2 SAD subgroups



Legend to Figure 3. Prevalence rates of Large Airways abnormalities, and Small Airways abnormalities in the full cohort of asthma participants (upper Figure), and according to Clinical SAD Group1 and Group2 (lower Figure). Prevalences are based on LLN (Lower Limit of Normal) and ULN (Upper Limit of Normal) values derived from the literature or from ATLANTIS controls without airway disease, noted with*. For abbreviations see Table 2.

Figure 4. Cross-Sectional Clinical SEM analyses of small airway function

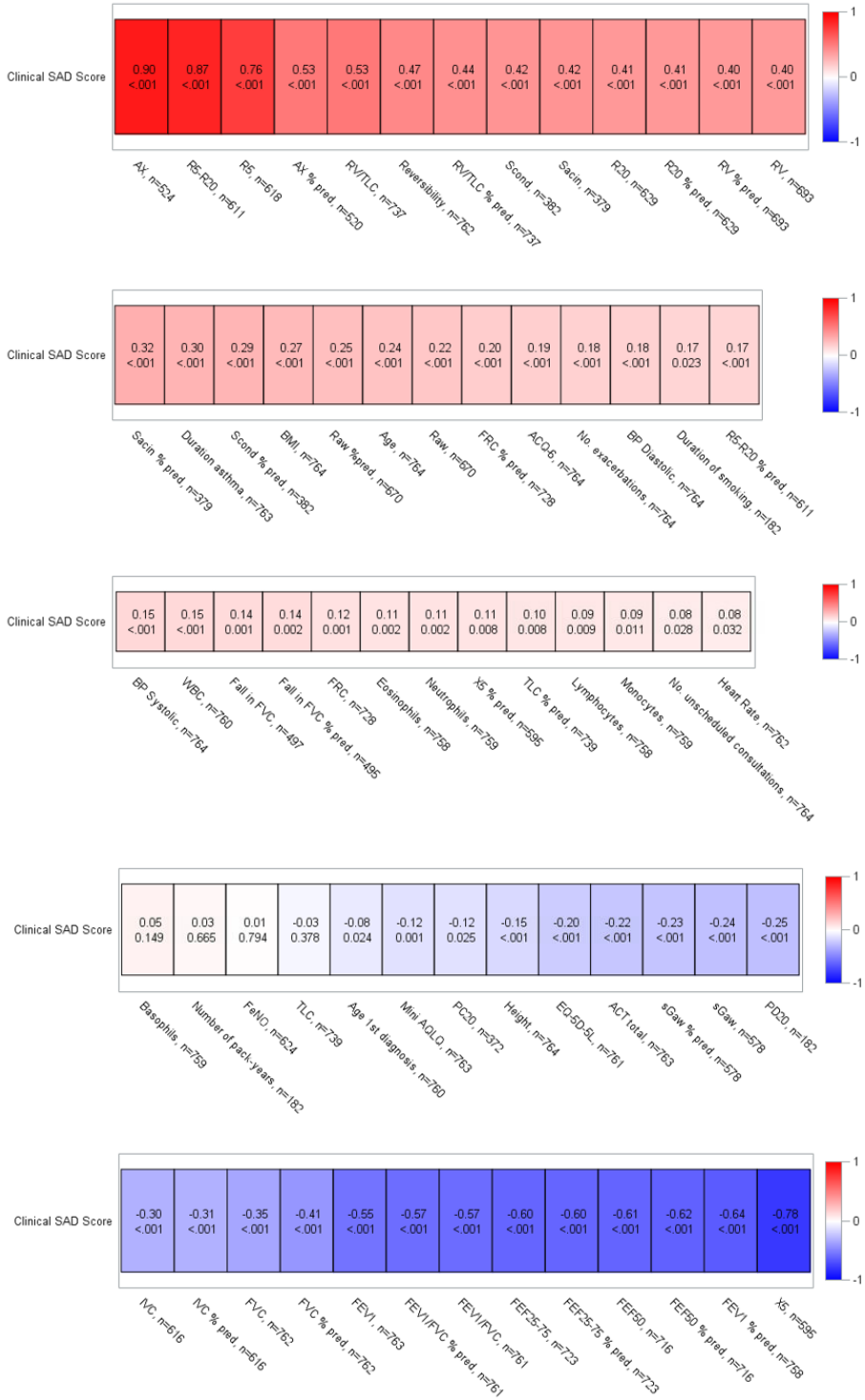


Legend to Figure 4. SAD=Small Airway Dysfunction.

The figure shows the results of Structural Equation Modeling (SEM). The model uses the measured variables presented in squares to define the three latent variables (Lung1, Lung2 and Lung3). The strength of the relationship of each measured variable to the underlying factor is expressed by the factor loading, presented in the Figure in dashed squares. Moreover, the numbers that are not presented in squares are the measured variable variances. The variable SAD is then constructed by a structural model that imputes the relations between these three latent variables (Lung1 loading 0.617, Lung2 loading 0.518 and Lung3 loading 0.981). Thus SEM modeling showed that SAD was built up by three latent variables, represented in circles (Lung1 loading 0.617, Lung2 loading 0.518 and Lung3 loading 0.981). The measured variables are presented in squares . IOS parameters R5-R20, X5 and AX (reflecting small-to-mid-sized airway obstruction/distensibility) loaded to the first latent variable (Lung1), FEF_{50} and FEF_{25-75} both corrected for FVC (reflecting small-to-mid-sized airway obstruction), to the second latent variable, while MBNW parameter Sacin (reflecting dysfunction in the most peripheral airways) loaded both to the first and second latent variable. The lung volume parameter RV/TLC % predicted (most peripheral airways dysfunction) and MBNW parameter Scond (dysfunction in small-medium size conducting airways) loaded to the third latent variable (Lung3). Please Note that Sacin loaded equally with 0.285 and 0.291 to latent variable Lung1 and Lung2 respectively. Please Note that Sacin loaded equally with 0.285 and 0.291 to latent variable Lung1 and Lung2 respectively. The numbers on the right hand

side represent the variance of the measures, i.e. variance in AX is 0.009, contrasting with the variance in RV/TLC % predicted being 0.738. Goodness of fit of the SEM model was evaluated through the following fit indices: Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), Tucker-Lewis Index (TLI). The closer CFI and TLI are to 1 and the closer RMSEA is to 0 the better is the model fit. The goodness of fit values (Supplemental methods) show there is good coherence of this model to SAD. Fall in FVC during hyperresponsiveness testing contributed to the model when analyzed in the subgroup of asthmatic participants who had undergone hyperresponsiveness testing (see also Supplement for model comparison).

Figure 5. Correlations of the Clinical SAD score of asthma participants with all parameters measured



Legend to Figure 5. For abbreviations see Table 2

Table 1. Parameters of SAD as presented in the SEM analyses of the study and their hypothetical location.

Physiologic parameters	
FEF_{25-75} (corrected for FVC)[12,13]	Obstruction measure in small-to-mid-sized airways and peripheral small airways at mid expiratory lung volume
FEF_{50} (corrected for FVC)[12,13]	Obstruction measure in small-to-midsized airways
RV/TLC[14,15]	Air trapping due to obstruction in both conducting small and peripheral airways
FRC[16]	Respiratory system resting volume as main determinant of whole airway static dimensions, and airway hysteresis
R5-R20[17]	Resistance of small-to-mid-sized airways
X5[17,18]	Reactance or distensibility of small-to-mid-sized conducting airways
AX[17,18]	Distensibility of small-to-midsized conducting airways
Scond[19]	Index of convectional ventilation heterogeneity in peripheral conducting airways
Sacin[20]	Index of diffusive ventilation heterogeneity in most peripheral pre-acinar/acinar airways
Fall in FVC at PC_{20} or PD_{20}[21,22]	Air trapping due to excessive bronchoconstriction or closure of small airways
CT parameters	
MLD ratio[25]	Ratio of mean lung density for inspiratory versus expiratory scans - a measure of air trapping due to lung parenchyma inspiratory distension in the supine position
Lung volume ratio[24]	Ratio of CT-derived lung volume for inspiratory versus expiratory scans - a measure of air trapping due to obstruction in both conducting small and peripheral airways in the supine position
VI-856[24]	The voxel index below 856 Houndsfield Units from the expiratory scans, an index of expiratory air trapping

Legend to Table 1. Numbers in superscript refer to references used.

Physiologic parameters	Interpretation

Spirometry	
FEF_{25-75} (corrected for FVC), L/s/L [13] and FEF_{50} (corrected for FVC), L/s/L [13]	FEFs at 25-75% interval, or at 50% of expired lung volumes are measurements of airflow obstruction in small-to-mid-caliber airways taken at low/mid expiratory lung volumes. When corrected for FVC, they are surrogate measures of the sizes of small-to-mid caliber airways relative to lung size, called dysanapsis. Dysanapsis is a characteristic favoring airways hyperresponsiveness.
Body plethysmography	
RV/TLC ratio, L/L [14]	Air trapping due to obstruction in both conducting small and peripheral airways
FRC, L [14]	Respiratory system resting volume as main determinant of whole airway static dimensions, and airway hysteresis
IOS	
R5-R20, kPa/L/s [15]	Respiratory Resistance of small-to-mid-sized conductive and peripheral airways
X5, kPa/L/s [15]	Respiratory System Reactance reflecting inertance and elasticity (capacitance), including small peripheral airways
AX, kPa/L [15]	Distensibility of the peripheral lungs (parenchyma + small peripheral airways)
MBNW	
Scond*VT, L^{-1} [16]	Index of convectional ventilation heterogeneity in peripheral conducting airways
Sacin*VT, L^{-1} [17]	Index of diffusive ventilation heterogeneity in most peripheral pre-acinar/acinar airways
Hyperresponsiveness	
Fall in FVC at PC_{20} or PD_{20}, % [18,19]	Air trapping due to excessive bronchoconstriction or closure of small airways
CT scan parameters	
MLD E/I ratio [25]	Ratio of mean lung density for inspiratory versus expiratory scans- a measure of air-trapping due to lung parenchyma inspiratory distension in the supine position
Lung volume ratio, cm^3 [22]	Ratio of CT-derived lung volume for inspiratory versus expiratory scans- a measure of air-trapping due to obstruction in both conducting small and peripheral airways in the supine position
VI-856, HU [21]	The voxel index < -856 Hounsfield Units from the expiratory scans, an index of expiratory air trapping

Legend to Table 1. Numbers in superscript refer to references used. IOS= impulse oscillometry; MBNW= Multiple breath nitrogen washout; CT= computed tomography, HU=Hounsfield Units

Table 2: Baseline clinical, physiologic and CT characteristics of asthma participants and controls without airway disease

Parameter	Asthma	Controls	P - value
	n=773	n=99	
Clinical characteristics			
Age, years	46 (34 ; 54)	41 (29 ; 52)	0.0107
Gender, female N (%)	450 (58)	56 (57)	0.754
Heart rate, bpm	71 (65 ; 78)	68 (61 ; 75)	0.0104
BP syst, mmHg	123 (114 ; 131)	120 (110 ; 130)	0.0109
BP diast, mmHg	80 (70 ; 84)	75 (68 ; 83)	0.0655
BMI, kg/m^2	26 (23 ; 30)	24 (21 ; 27)	<0.001
Atopy (Phadiatop), N (%)	454 (81)	39 (46)	<0.001
FeNO, ppb	25 (16 ; 38)	18 (11 ; 26)	<0.001
Ex-smoker, N (%)	156 (20)	19 (19)	0.393
Current Smoker, N (%)	27 (4)	1(1)	
Eosinophils, 10^9 /L	0.2 (0.1 ; 0.4)	0.1 (0.1 ; 0.2)	<0.001
Neutrophils, 10^9 /L	3.7 (3.0 ; 4.7)	3.3 (2.7 ; 4.4)	0.010
PC_{20}, mg/mL	1.25 (0.4 ; 4.2)	15.23 (16.0 ; 16.0)	<0.001
PD_{20}, mg	0.11 (0.0 ; 0.6)	1.86 (2.0 ; 2.0)	<0.001
Moderate-severe hyperresponsiveness, N (%)	271 (48.4)	0 (0.0)	<0.001
Fall in FVC, %	17 (12 ; 22)	4 (1 ; 8)	<0.001
Lung Physiology characteristics (%predicted)			
FEV_1, %predicted	82.7 (69.9 ; 93.8)	100.4 (91.6 ; 107.3)	<0.001
Change FEV_1, %predicted	7.6 (4.1 ; 12.7)		
FEV_1/FVC, %predicted	85.8 (76.5 ; 93.9)	98.2 (93.8 ; 102.7)	<0.001
IVC, %predicted	99.0 (18.21)	109.7 (15.28)	<0.001
FEF_{50}, %predicted	62.0 (43.2 ; 84.1)	102.0 (84.8 ; 117.3)	<0.001
FEF_{25-75}, %predicted	56.6 (37.6 ; 75.6)	90.7 (75.6 ; 108.1)	<0.001

RV, %predicted	117.1 (98.4 ; 138.9)	95.6 (87.0 ; 115.7)	<0.001
TLC, %predicted	104.9 (95.9 ; 115.5)	104.8 (96.7 ; 112.5)	0.62~~16~~
RV/TLC, %predicted	106.1 (91.6 ; 125.8)	92.5 (80.6 ; 109.6)	<0.001
FRC, %predicted	108.7 (93.4 ; 126.7)	107.6 (91.9 ; 121.4)	0.42~~19~~
Raw, %predicted	143.0 (91.4 ; 231.1)	77.6 (62.9 ; 99.5)	<0.001
sGaw, %predicted	60.5 (42.5 ; 94.7)	85.0 (61.3 ; 124.6)	<0.001
R20, %predicted	114.6 (97.4 ; 134.9)	96.5 (84.7 ; 110.2)	<0.001
R5-R20, %predicted	278.6 (91.2 ; 640.9)	69.5 (0.0 ; 161.7)	<0.001
X5, %predicted	130.4 (94.4 ; 184.7)	94.6 (77.6 ; 119.7)	<0.001
AX, %predicted	209.3 (95.0 ; 510.0)	66.1 (49.9 ; 108.0)	<0.001
Scond*VT, %predicted	180.5 (100.7 ; 305.3)	95.6 (44.8 ; 149.6)	<0.001
Sacin*VT, %predicted	107.2 (76.7 ; 154.8)	94.1 (61.6 ; 129.8)	0.01~~4~~
CT Scan characteristics			
MLD Inspiratory, HU	-837.93 (-856.95 ; -811.97)	-839.89 (-853.81 ; -812.76)	0.65~~1~~
MLD Ratio E/I	0.83 (0.77 ; 0.88)	0.80 (0.73 ; 0.87)	0.08~~1~~
VI-856	7.82 (2.5; 19.5)	7.83 (1.5; 15.5)	0.35~~47~~
Lung Volume Ratio	0.50 (0.43 ; 0.60)	0.47 (0.38 ; 0.56)	0.15~~5~~6
Percentile 15 Inspiratory	-921 (-935;-904)	-929 (-940;-899)	0.46~~3~~
Median LA/BSA Inspiratory	10.4 (2.93)	11.4 (2.83)	0.03~~27~~
Median LA Inspiratory	19.0 (15.7 ; 23.3)	21.3 (18.5 ; 25.6)	0.01~~3~~
Pi10 Inspiratory	7.21 (6.59 ; 7.77)	6.70 (6.28 ; 7.84)	0.07~~3~~
Po20 %WA Inspiratory	7.41 (6.67 ; 8.50)	7.33 (6.42 ; 9.02)	0.73~~2~~

Legend to Table 2: All parameters are presented as Mean (standard deviation), Median (Quartile1 - Quartile 3), or N (%) as appropriate. BP= Blood Pressure, Syst=Systolic, BMI= Body Mass Index, FeNO=Fraction of exhaled Nitric Oxide, WBC=White Blood Cell, RV= Residual Volume, FRC=Functional Residual Capacity, PC=Provocative Concentration, PD=Provocative Dose, PC_{20} and PD_{20} = the provocative concentration and dose, respectively, that cause a 20% fall in FEV_1 from baseline FEV_1 during methacholine challenge, Fall in FVC, % fall in FVC at PC_{20} or PD_{20}; FEV_1=Forced Expiratory Volume in the 1st second, FVC= Forced Vital Capacity, FEF_{50}=Forced Expiratory Flow at 50% of FVC, IVC=Inspiratory Vital Capacity, FEF_{25-75}= Forced Expiratory Flow at 25%-75% of FVC,RV= Residual Volume, TLC=Total Lung Capacity, FRC= Functional residual Capacity, Raw- airway resistance, sGaw= specific

airway conductance, R5-R20= Peripheral Airway Resistance, X5= Resistance at 5 Hz, AX= Area of Reactance, Scond*VT= ventilation inhomogeneity in the conductive zone of the lungs, Sacin*VT= Ventilation inhomogeneity of the acinar zone of the lungs, CT= Computed tomography, MLD Ratio E/I= Mean Lung Density Expiratory to Inspiratory ratio, E=Expiratory, I=Inspiratory, LA= Lumen Area (mm^2), BSA= Body Surface Area (m^2), VI-856= Voxel index at -856 Hounsfield Units.

Table 3 Characteristics of asthma participants

Parameter	
GINA 1, N (%)	135 (17.5)
GINA 2, N (%)	85 (11.0)
GINA 3, N (%)	207 (26.8)
GINA 4, N (%)	300 (38.8)
GINA 5, N (%)	46 (6.0)
Medication use	
SABA, N (%)	671 (86.8)
Short acting anticholinergics, N (%)	9 (1.2)
LABA, N (%)	86 (11.1)
ICS, uncombined N (%),	183 (23.9)
Extra-fine ICS, N (%)	58 (7.5)
Non-extra-fine ICS, N (%)	127 (16.4)
ICS mean daily dose (BDP equivalent), µg	669 (446)
ICS/LABA, N (%)	460 (59.5)
ICS/LABA mean daily dose (BDP-equivalent), µg	882 (634)
Extra-fine ICS/LABA, N (%)	124 (16.0)
Non-extra-fine ICS/LABA, N (%)	336 (43.5)
Oral corticosteroids, N (%)	22 (2.8)
Oral corticosteroids mean daily dose, mg	7.5 (5.0 ; 20.0)
Montelukast, N (%)	144 (18.6)
LAMA, N (%)	29 (3.8)
Biologics, N (%)	32 (4.1)
Duration of disease, years	16.7 (5.6 ; 29.3)
Age 1st diagnosis <18 years, %	39
Unscheduled consultations past 12 months, N	0.3 (1.4)
Exacerbations past 12 months, N	0.2 (0.6)
>1 exacerbation past 12 months, %	14

ACT, total score	21.0 (18.0 ; 24.0)
ACT < 15, %	13
ACQ-6, total score	0.8 (0.3;1.5)
ACQ-6 > 1.25, %	33
EQ-5D-5L, VAS score	80.0 (70.0 ; 90.0)
Mini AQLQ, total score	5.6 (4.7 ; 6.3)

Legend to Table 3. Data are presented as N (%) or Median (Q1 to Q3 ranges) as appropriate. ACT=Asthma Control Questionnaire, ACQ-6= Asthma Control Questionnaire-6, EQ-5D-5L= Standardized measure of health status descriptive system, Mini AQLQ= Mini Asthma Quality of Life Questionnaire. Number of exacerbations and unscheduled consultations are based on the past 12 months. The daily dose of ICS (inhaled corticosteroids) is expressed in BDP equivalents, µg/day

Table 43. Prevalence rates (%) of abnormal SAD parameters (>ULN or <LLN) according to GINA stages

Parameter, %	**GINA 1**	**GINA 2**	**GINA 3**	**GINA 4**	**GINA 5**
FEF_{25-75}	41.4	43.0	50.5	54.5	80.4
FEF_{50}	37.3	49.4	54.1	55.3	75.0
% fall FVC	71.7	67.9	75.2	72.7	84.2
RV/TLC	14.0	16.3	19.3	28.1	31.1
FRC	16.2	23.4	19.1	24.5	27.3
R5-R20	29.9	40.0	36.5	50.5	70.6
AX	32.4	34.4	35.4	49.2	67.7
X5	22.8	31.8	28.5	33.2	53.1
Scond	20.5	20.0	30.0	33.3	63.6
Sacin	12.3	17.8	18.5	20.5	40.9

Legend to Table 3. for abbreviations see Table 2. GINA severity was based on past treatment used. Note that the highest prevalence of SAD is always in GINA5, the lowest prevalence across all GINA stages is with Sacin.

Table 54. Relationship of lung physiology variables with number of exacerbations and unscheduled consultations

Number of exacerbations

Independent variables included in the final model	Coefficient	P-value type 1	P-value type 3
$FEF_{25-75,}$ L/s corrected for FVC	-1.226	0.034	
R5-R20, kPa/L/s	2.894	0.010	
Raw, kPa*s/L	-2.286	0.014	
RV/TLC, ratio	2.773	0.0438	
sGaw, 1/kPa*s	-0.316	0.0327	
Height, cm	-0.053	<.001	
PC_{20} and PD_{20} categories – very mild vs normal	-1.058	0.0217	0.006
PC_{20} and PD_{20} categories - mild vs normal	-1.624	<.001	
PC_{20} and PD_{20} categories - moderate-severe vs normal	-1.212	0.0104	
Sex - Female vs Male	0.717	0.0326	

Number of unscheduled consultations due to worsening symptoms

Independent variables included in the final model	Coefficient	P-value type 1	P-value type 3
FEV_1 , L	0.647	<.001	
FRC , L	-0.425	0.0107	
RV/TLC, ratio	4.659	0.0101	
sGaw , 1/kPa*s)	-0.466	<.001	
PC_{20} and PD_{20} categories – very mild vs normal	-0.999	0.0104	0.023
PC_{20} and PD_{20} categories - mild vs normal	-0.888	0.0108	
PC_{20} and PD_{20} categories - moderate-severe vs normal	-0.792	0.012	
Sex (male/female) - Female vs Male	0.647	0.023	

Legend to Table 4. MBNW parameters were not used, since this would restrict the number of asthmatics to be analyzed (see Methods). P-value type 3 assesses the statistical difference in hyperresponsiveness severity stages. The coefficients are per 1 unit increase in each parameter. As example: the estimate of R5-R20 (kPa/L/s) for exacerbations is 2.894, one needs to calculate exp(2.894) = 18.065 and this means that for 1-unit increase of R5-R20 the mean number of exacerbations will increase by a factor of 18.07, holding other variables constant. Mild hyperresponsiveness means a

higher PD_{20} or PC_{20} value. Patients with more severe hyperresponsiveness have more frequent exacerbations and unscheduled consultations. For abbreviations see Table 2.

Table 6. Clinical characteristics of asthma participants in Clinical SAD Group1 and Clinical SAD Group2

Parameter	Group1 (n=452)	Group2 (n=312)	P-value
Clinical SAD score	-0.256 (-0.34;-0.16)	0.284 (0.12;0.56)	<0.001
Age, years	43 (30;53)	50 (40;58)	<0.001
Gender, female N (%)	257 (57)	186 (60)	0.45~~48~~
Heart rate, bpm	70 (64;77)	72 (65;80)	0.02~~3~~
BP syst, mmHg	120 (110;130)	125 (117;135)	<0.001
BP diast, mmHg	78 (70;82)	80 (72;87)	<0.001
BMI, kg/m^2	25 (22;28)	28 (25;32)	<0.001
Atopy, N (%)	262 (81)	187 (79)	0.53~~1~~
FeNO, ppb	24 (16;37)	25 (16;39)	0.42~~4~~
Ex-smoking, N (%)	90 (20)	65 (21)	0.47~~4~~
Duration smoking, years	10 (5.1;16.7)	14 (8.0;20.0)	0.02~~0~~
GINA 1/2, N (%)	157 (35)	60 (9)	<0.001
GINA 3, N (%)	135 (30)	70 (22)	<0.001
GINA 4/5, N (%)	160 (35)	182 (58)	<0.001
ICS uncombined, N (%)	98 (22)	83 (27)	0.12~~16~~
ICS/LABA, N (%)	254 (56)	202 (65)	0.02~~18~~
ICS dose, BDP equivalence	603.2 (384.9)	739.9 (482.5)	0.08~~79~~
ICS/LABA dose, BDP equivalence	818.8 (563.1)	959.6 (710.8)	0.07~~88~~
Oral corticosteroids, N (%)	8 (1.8)	14 (4.5)	0.03~~27~~
Eosinophils, 10^9 /L	0.21 (0.12;0.35)	0.26 (0.16;0.40)	<0.001
Neutrophils, 10^9 /L	3.50(2.88;4.47)	3.90(3.07;4.91)	<0.001
FEV_1, %predicted	90.2 (80.1 ; 98.4)	70.1 (58.8 ; 81.8)	<0.001
Change FEV_1, %predicted	6.5 (3.6 ; 9.9)	10.2 (5.5 ; 14.9)	<0.001
FEV_1/FVC, %predicted	90.1 (83.4 ; 96.6)	78.3 (70.5 ; 86.0)	<0.001
FEF_{50}, %predicted	75.2 (59.1 ; 94.8)	44.4 (31.5 ; 59.7)	<0.001
IVC, %predicted	103.3 (18.0)	93.1 (17.0)	<0.001
FEF_{25-75}, %predicted, N (%)	66.6 (51.7 ; 86.9)	37.7 (27.8 ; 52.2)	<0.001

RV, %predicted	108.9 (92.7 ; 127.2)	134.2 (110.9 ; 158.8)	<0.001
TLC, %predicted	104.3 (95.7 ; 114.0)	105.9 (95.9 ; 116.9)	0.2439
FRC, %predicted	107.3 (91.7 ; 123.0)	111.2 (94.8 ; 129.9)	0.011
Raw, %predicted	110.1 (81.4 ; 167.8)	192.3 (139.6 ; 309.3)	<0.001
sGaw, %predicted	66.5 (47.4 ; 105.1)	47.0 (33.9 ; 72.4)	<0.001
R20, %predicted	107.8 (92.2 ; 125.7)	126.3 (109.7 ; 147.9)	<0.001
R5-R20, %predicted	129.6 (29.0 ; 304.0)	636.3 (378.2 ; 1065.0)	<0.001
X5, %predicted	109.1 (80.9 ; 140.5)	199.0 (151.6 ; 254.6)	<0.001
AX, %predicted	115.3 (65.3 ; 198.3)	613.6 (384.7 ; 868.3)	<0.001
Scond*VT, %predicted	144.6 (75.9 ; 239.7)	245.2 (161.7 ; 392.1)	<0.001
Sacin*VT, %predicted	93.1 (70.6 ; 127.0)	140.8 (95.8 ; 190.5)	<0.001
No. unscheduled consultations , N	0.15 (0.57)	0.50 (2.08)	0.001
No. exacerbations, N	0.16 (0.52)	0.29 (0.76)	0.002
>= 1 exacerbation, N (%)	50 (11.1)	59 (18.9)	0.002
Duration of disease, years	11.6 (4.4 ; 24.5)	21.5 (9.4 ; 35.0)	<0.001
Age at 1st **Diagnosis, years**	25 (10 ; 41)	22 (7 ; 41)	0.131
Age at 1st **Diagnosis < 18 years, N(%)**	162 (36.2)	134 (42.9)	0.0659
ACT, total score	22.0 (19.0 ; 24.0)	20.0(17.0 ; 23.0)	<0.001
ACT score ≤ 15, N (%)	40 (8.9)	60 (19.2)	<0.001
ACQ-6, total mean score	0.66 (0.2 ; 1.3)	1.00 (0.5 ; 1.8)	<0.001
ACQ-6 score ≥ 1.25, N (%)	124 (27.4)	126 (40.4)	<0.001
EQ-5D-5L, VAS score	83.0 (75.0 ; 90.0)	80.0 (70.0 ; 90.0)	<0.001
Mini-AQLQ, total score	5.7 (4.8;6.4)	5.5(4.5;6.3)	
CT Scan characteristics			
MLD Inspiratory, HU	-844.53(-859.56 ; -815.71)	-831.65(-854.46 ; -808.68)	0.0986
MLD Ratio E/I	0.82 (0.76 ; 0.87)	0.84 (0.78 ; 0.90)	0.0107
VI-856	6.96 (1.92 ; 18.27)	9.54 (3.18 ; 21.30)	0.0768
Lung Volume Ratio	0.49 (0.41 ; 0.56)	0.51 (0.45 ; 0.62)	0.008
Percentile 15 Inspiratory	-922.33 (-937.51 ; -906.97)	-917.72 (-930.20 ; -900.38)	0.054
Median LA/BSA Inspiratory	10.95 (2.66)	9.67 (3.08)	<0.001

Median LA Inspiratory	20.37 (17.32 ; 23.47)	17.82 (14.59 ; 22.08)	<0.001
Pi10 Inspiratory	7.12 (6.54 ; 7.77)	7.28 (6.59 ; 7.78)	0.64~~1~~
Po20 %WA Inspiratory	7.49 (6.71 ; 8.52)	7.27 (6.57 ; 8.41)	0.4658

Legend to Table 6. Data are presented as N (%), Mean (SD) and Median (interquartile ranges) as appropriate; for abbreviations see Table 2~~1~~ and Table 3.

Supplement belonging to

Exploring the relevance and extent of small airways dysfunction in asthma: AssessmenT of smalL Airways involvemeNT In aSthma, the ATLANTIS study

Postma DS, Brightling C, Baldi S, Van den Berge M, Fabbri L, Gagnatelli A, Papi A, Van der Molen T, Rabe K, Siddiqui S, Singh D, Nicolini G, Kraft M and the ATLANTIS study group*

Collaborators

* Badorrek P, Boersma W, Broeders M, Chetta A, Cukier A, D'Amato M, Djukanovic R, Foschino MP, Gessner C, Hanania N, Martin R, Milleri S, Olivenstein R, Paggiaro P Pizzichini E, Plaza Moral V, Scichilone N, Schilz R, Spanevello A, Stelmach R, Vroegop J, Usmani OS, Zhang Q.

Methods

Participants were recruited in 29 worldwide sites: Brazil (3), Canada (1), China (1), Germany (3), Italy (8), the Netherlands (4), Spain (1), UK (4), USA (4). Participants gave informed consent separately for the full study and the optional measurements (e.g. Computed Tomography (CT) scan, bronchial biopsies). All measurements were performed according to international guidelines, if available (e.g. spirometry, body plethysmography), or pre-specified, standardized procedures in references or as described in detail below. Some measurements were performed in a subgroup of patients in selected sites (e.g. Fraction of exhaled Nitric Oxide (FeNO), oscillometry system (IOS), CT).

The baseline visit was divided into three days to allow all the different tests being performed without conflict. Day 1 included measurements in the following order: healthcare resource consumption assessments (asthma participants only); Asthma Control Test (ACT), 6-items Asthma Control Questionnaire (ACQ-6) and mini-Asthma Quality of Life Questionnaire (mini-AQLQ); Small Airways Dysfunction Tool (SADT) and EuroQol-5D-5L (EuroQol-5 dimensions-5Levels); bronchial hyperresponsiveness questionnaire (BHQ); FeNO; Methacholine challenge test; nasal brushing. Day 2 included measurements in the following order: pre-bronchodilator IOS; pre-bronchodilator Multiple Breath Nitrogen Washout test (MBNW); pre-bronchodilator lung volumes measurement with body box; pre-bronchodilator spirometry; only in asthma participants the following: administration of 4 puffs (4 × 100 µg) of salbutamol by pressured metered dose inhaler plus spacer; post-bronchodilator IOS; post-bronchodilator MBNW; Post-bronchodilator lung volumes measurement with body box; Post-bronchodilator spirometry; CT scan. Sites performing bronchoscopy with endobronchial and transbronchial biopsies had a further separate third visit.

Pulmonary function tests

Lung function measurements and daily calibration of the equipment was performed according to the recommendation of the Official Statement of the European Respiratory Society and American Thoracic Society[1]. Predicted values, where available, were calculated according to the formulas reported by Quanjer et al.[2]. Throughout the study, the clinic visits and the lung function measurements started in the morning, approximately at the same time of the day for each patient and after appropriate washout from bronchodilators, i.e. 6 hours for short acting β2-agonists (SABA); 12 hours (8 hours in patients with severe asthma defined as step 4 or 5 of treatment according to GINA 2014 www.ginasthma.org) for short acting antimuscarinic agents (SAMA); 12 hours for long acting β2-agonists twice daily (LABA); 72 hours (24

hours in patients with severe asthma defined as step 4 or 5 of treatment according to GINA 2014 www.ginasthma.org) for long-acting β2-agonists once daily (LABA) and long-acting antimuscarinic agents (LAMA) once daily.

Airway hyperresponsiveness was measured with the two-minute tidal breathing method or 5-breath dosimeter method according to the published international guidelines[3,4]. As example, the tidal breathing method consists of the inhalation of increasing concentrations of methacholine by using a nebulizer with a two-way valve that allows a controlled amount of material to be delivered during continuous nebulization while the patient breathes quietly (tidal breathing) for two minutes. This procedure is repeated with increasing concentrations of methacholine until the Forced Expiratory Volume in one second (FEV_1) decreases by at least 20% from baseline value or until the highest concentration of methacholine (16 mg/mL) has been delivered. At the same time, the corresponding fall in Forced Vital Capacity (FVC) is recorded. Positive airway hyperresponsiveness to methacholine was defined as the provocative concentration or provocative dose when the FEV_1 falls with 20% from baseline FEV_1, i.e. PC_{20} < 8 mg/mL or PD_{20}< 0.7 mg for those subjects not using inhaled corticosteroids (ICS) and PC_{20} < 16 mg/mL or PD_{20} < 1.4 mg for subjects using ICS respectively.

For analyses, we stratified asthma patients for severity of airway hyperresponsiveness as follows: 1) normal PC_{20} and PD_{20} > 16 mg/ml and >1 mg respectively, 2) very mild 4-16 mg/ml and 0.6-0.10 mg, 3) mild 1-4 mg/ml and 0.6-1.0 mg, and 4) moderate-severe < 1 mg/ml and < 0.3 mg respectively.

Body plethysmography was performed according to standard procedures[2]. Residual Volume (RV), RV divided by Total Lung Capacity (RV/TLC), Functional Residual Capacity (FRC), airway Resistance (Raw), specific airway conductance (sGaw) were used as parameters in the analyses.

Impulse oscillometry system (IOS) was performed according to the standard procedures[5] only if the instrument was locally available at the site (n= 22 out of 29 centers).

MBNW was performed with a standardized instrument provided to all sites: EXHALYZE-R D, according to previously published standard procedures[6]. In short, ventilation distribution inhomogeneity was assessed during tidal breathing from FRC, by examining inert gas clearance over a series of breaths. The indices of ventilation heterogeneity in the peripheral regions of the lung, where gas transport occurs predominantly across a pressure gradient through convection [ventilation heterogeneity in convection-dependent airways (Scond)] and across a concentration gradient through diffusion [ventilation heterogeneity in diffusion-dependent airways (Sacin)], were derived.

Questionnaires

Asthma control was measured with the ACT and the ACQ-6, whereas asthma-related quality of life was assessed with the mini-AQLQ[7,8]. Health status was assessed with EuroQol-5D-5L[9].

Healthcare resource consumption

Healthcare resource consumption in the past 12 months was assessed by the numbers of 1) unscheduled consultations for asthma (without hospitalization) defined as: the need for a visit (medical specialist or general practitioner) due to symptoms requiring treatment and 2) exacerbations defined by the use of an antibiotic and/or systemic corticosteroid course ($\geq$ 3 days). The other health care utilization questions were not analyzed, due to very low prevalences (asthma-specific hospital admissions (number and length), asthma-specific emergency room or urgent care visits; unscheduled tests for asthma (without hospitalization).

Blood analyses

Blood was collected for total and differential cell counts as performed in the local laboratories.

FeNO

FeNO was performed only if the instrument was locally available at the site (22 sites out of 29). Depending on the specific equipment available at the site, FeNO was measured as standard single flow (50ml/s) or multiple flows. FeNO measurements were performed, according to international guidelines before spirometry, at constant flow (50 mL/s) in triplicate by using an electrochemical/chemiluminescent analyzer. Exhaled NO levels were expressed as parts per billion (ppb). The mean of 3 values obtained within 10% of each other was used to calculate FeNO result.

If available, measurements at multiple constant flows (50, 100, 150, and 350 mL/s) was performed. At each flow, the mean of 3 values obtained within 10% of each other was used to calculate bronchial (J_{NO}) and alveolar NO (C_{alv}). The contributions of the bronchi (bronchial NO flux) and the alveoli (alveolar NO concentration) to FeNO was derived from regression analysis, with NO output as the dependent and exhalation flow rate as the independent factor. The slope and intercept of the regression line are approximate values of alveolar NO concentration and bronchial NO flux, respectively.

Computed Tomography

Volumetric whole lung scans were obtained using a standardised protocol for each scanner manufacturer and model to approximate to the reference scanner site (Leicester, Siemens Sensation 16 scanner [16 x 0.75 mm collimation, 1.5 mm pitch, 120 kVp, 40 mAs, 0.5 seconds rotation time and scanning field of view of 500 mm]). The scans were obtained at full inspiration (near TLC) and at the end of expiration (near FRC). All subjects were coached in

the breath holding techniques, and practiced breath holding, immediately prior to scanning. All asthmatics were scanned within 60 minutes of receiving 400 micrograms of salbutamol via a spacer. Images were reconstructed with a slice thickness of 0.75 mm at a 0.5 mm interval using B35f kernel for the reference scanner or similar algorithm. Post processing was performed on semi-automated software, Apollo (VIDA Diagnostics, Iowa).

QCT parameters obtained, with excellent inter-observer variability[10], included morphometry, measured in mm^2, Lumen Area (LA), Total Area (TA), Wall Area (WA) $(TA - LA)$ and percentage Wall Area (%WA) $\left(100\ x\left(\frac{(TA-LA)}{TA}\right)\right)$. Air-trapping measures were Mean Lung Density (MLD), the mean value of CT numbers distribution, measured upon inspiration (MLD_I) , and upon expiration (MLD_E), and MLD Expiratory to Inspiratory ratio ($MLD_{E/I}$) measured in Hounsfield Units (HU) and Relative Voxel Change (RVC) $Exp\big((VI - 856) - (VI - 950)\big) - Insp\big((VI - 856) - (VI - 950)\big)$. The Perc15 was measured in Hounsfield Units (HU), i.e. the value below which the 15% of voxels with the lowest density are distributed. Fractal dimensions of the low attenuation clusters on inspiratory scans (LAC-D -950) and on expiratory scans (LAC-D -856) were also measured. All morphometry measures were corrected for Body Surface Area mm^2/m^2 (BSA) $\left(\sqrt{\frac{height\ (cm)x\ weight\ (kg)}{3600}}\right)$. CT scans were excluded for the following reasons: i) deviations in the CT acquisition protocol, ii) lung density was greater in the inspiratory compared to expiratory scans and iii) CT and body plethysmography lung volumes were compared and for scans where the volumes were discrepant from the mean by > 3 SD. Density measures were corrected to account for scanner manufacturer and model variability. A representative example of an inspiratory and expiratory scan, airway reconstruction from the inspiratory scan and densitometry maps from both the inspiratory and expiratory scans are as shown in Supplemental Figure 1.

Statistical analyses

A total of 800 subjects were considered sufficient to estimate all necessary parameters of the structural equation model (SEM). Assuming a Subjects-To-Variables (STV) ratio of 20:1 [11,12] it was possible to include up to 13 factors of influence (3 parameters per factor) into the model. Assuming a small variability in the parameters of interest of healthy subjects, a number of 100 healthy volunteers was considered sufficient. The sample size for patients undergoing CT-scans was calculated based on the prevalence of patients with SAD expected to be approximately 20%, an agreement between classification of SAD based on the SEM and CT-scan $\geq$ 0.8 (Cohen's kappa), a Type-I-error of 0.05 and a Power of $\geq$ 80%. Following the algorithm given by Cantor[13], a sample size of $\geq$ 528 asthmatics valid for CT-scan and SEM evaluation was calculated. Moreover, Approximately 50 controls were considered sufficient to provide reference normal values for CT scan airways geometry and densitometry.

Primary and secondary analyses were performed on the evaluable set. This analysis set comprised all enrolled subjects, independently of any possible protocol deviation, who underwent visit 1 with at least one study endpoint related to the primary analysis noted.

SEM model

Structural equation modeling was applied in asthma. As small airways dysfunction cannot be directly observed and measured, we inferred its unobserved or ''latent'' constructs by modeling them from the physiological and CT data we collected on related and directly measurable variables, which are the "effects" of the latent constructs itself. The following variables reflecting Small Airways Dysfunction and both Small and Large Airways Dysfunction were considered in the SEM model: % fall in FVC at PC_{20} or PD_{20}, R5-R20 (small-to-mid-sized airway resistance with IOS), X5 (resistance at 5Hz with IOS), AX (Airway reactance with IOS), Scond, Sacin, RV % predicted, RV/TLC % predicted, FRC

%predicted, Forced Expiratory Flow at 50% of FVC $(FEF)_{50}$/FVC, FEF_{25-75}/FVC, FEV_1 % predicted, FEV_1/FVC, IVC % predicted, PC_{20}.

The correlation matrix was computed to evaluate the correlations among observed variables. Summary statistics were presented. High correlations among the observed variables indicated the presence of underlying latent variables. An exploratory factor analysis for observed variables was performed in order to identify the underlying SAD factor structure. The final underlying SAD factor structure identified at the previous step was tested by specifying a confirmatory factor model.

1. Causal model

Once that the measurement model was set up, each causal relationship specified in the initial path diagram was added and tested one at time to build the final structural equation model.

2. Small Airways Dysfunction classification

Once the measurement model was set and fit the data properly, it was considered completed and it was used to classify each patient in the SAD groups, by using a model-based clustering. The initial assumption of two groups (based on clinical hypotheses) was compared to different numbers of groups, by means of posterior fit statistics such as AIC, BIC and Entropy. The Log-likelihood and the number of parameters were presented as well.

3. Agreement evaluation

A SAD classification was obtained by considering CT Scan as well. A model-based clustering was used to classify each asthmatic patient in two groups identifying SAD or not SAD, by using the CT Scan data reflecting Small Airways Dysfunction (MLD ratio, Lung Volume ratio, VI -856).

The classification and the scores derived from Clinical SAD model were compared with the one derived from CT-scan in order to evaluate the rate of agreement, using Cohen's kappa coefficient (K) and Pearson's correlation[14].

The detection of outliers in the SEM analysis was based on log-likelihoods of each observation in Full-Information Maximum-Likelihood (FIML) estimation. An individual with a high/extreme log-likelihood value with a disproportionate impact on the model was considered as outlier.

The following identification rules and estimation procedures / methods were considered in this analysis. Identification constraints of the scale and measurement units were taken into account for the latent variables. The estimation method used for all the analysis was the Robust Maximum likelihood estimation method that takes properly into account the different nature of the observed variables (binary, continuous, ordinal, count). When it resulted computational unfeasible the Robust Weighted Least Square estimation method was used alternatively.

Goodness of fit of the SEM model was evaluated through the following fit indices: Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), Tucker-Lewis Index (TLI). The closer CFI and TLI are to 1 and the closer RMSEA is to 0 the better is the model fit. These summary statistics (with the relative p-value, if applicable), together with the unstandardized and standardized parameter estimates (with the relative p-value), and the R-Square for each endogenous variable / factor if available, were computed for each part of the model.

The final clinical SAD model identified with cross-sectional data (visit 1) was tested also at visit 2 (6 months after the baseline visit) and visit 3 (12 months after the baseline visit). The final model structure was confirmed at each visit and the final longitudinal SEM model was

estimated. The clinical SAD groups at each visit were derived through a Latent Transition Analysis based on the longitudinal SEM model.

Multiple regressions

The relationships between lung physiology variables and healthcare consumption in asthmatics were analyzed using a Poisson regression model for each healthcare resource consumption variable. Only variables with a number of missing values approximately lower than 50% were considered in the analysis. The model included the healthcare resource consumption variable as dependent variable and all the lung physiology variables as independent variables. The overdispersion parameter based on the deviance was considered in order to account for between-patient variability and standard errors were estimated allowing for extra-Poisson variation. The backward elimination procedure was applied to identify the final model.

Predicted, Lower Limit of Normal (LLN) and Upper Limit of Normal (ULN) equations

Continuous prediction equations and their lower limits of normal for physiologic variables were computed using the following prediction equations. For variables such as FEV_1/FVC, FEV_1, the Quanjer regression equations[2] were used to derive the predicted value and the lower limit of normal (LLN) for each subject. For the variables such as RV, RV/TLC, FRC, IVC regression equations were used to derive the predicted value and the lower limit of normal (LLN) and upper limit of normal (ULN) for each subject.

For IOS and MBNW variables such as R20, R5-R20, X5, AX, Scond, and Sacin and FEF_{50} and % fall FVC, the regression models are presented in Supplemental Table 1. They were estimated with the study data from controls without airway disease in order to obtain the predicted, LLN and ULN value for each parameter as appropriate.

Supplemental Table 1. Summary equations for predicted values

Variable	Unit	Regression equation for predicted value	RSD
		Male	
R20	kPa/L/s	0.606347 – (Age x 0.000713) – (Height x 0.001702)	0.018575
R5-R20	kPa/L/s	0.270439 + (Age x 0.000285) – (Height x 0.001378)	0.039838
X5	kPa/L/s	– 0.473195 – (Age x 0.000867) + (Height x 0.002408)	0.040651
AX	kPa/L/s	2.426203 + (Age x 0.004599) – (Height x 0.013162)	0.326243
Scond	VT, L^{-1}	0.047168 + (Age x 0.000324) – (Height x 0.000245)	0.018575
Sacin	VT, L^{-1}	0.286222 + (Age x 0.000899) – (Height x 0.001158)	0.047755
FEF_{50}	L/sec	2.168062 – (Age x 0.035548) + (Height x 0.019211)	0.775259
Fall in FVC	%	– 24.93070 + (Age x 0.008664) + (Height x 0.156234)	4.424367
		Female	
R20	kPa/L/s	0.606347 – (Age x 0.000713) – (Height x 0.001702) + 0.040388	0.018575
R5-R20	kPa/L/s	0.270439 + (Age x 0.000285) – (Height x 0.001378) – 0.037831	0.039838
X5	kPa/L/s	– 0.473195 – (Age x 0.000867) + (Height x 0.002408) – 0.007246	0.040651
AX	kPa/L/s	2.426203 + (Age x 0.004599) – (Height x 0.013162) – 0.088393	0.326243
Scond*VT	L^{-1}	0.047168 + (Age x 0.000324) – (Height x 0.000245) + 0.001518	0.018575
Sacin*VT	L^{-1}	0.286222 + (Age x 0.000899) – (Height x 0.001158) – 0.041527	0.047755
FEF_{50}	L/sec	2.168062 – (Age x 0.035548) + (Height x 0.019211)	0.775259
Fall in FVC	%	– 24.93070 + (Age x 0.008664) + (Height x 0.156234) +4.498172	4.424367

Legend to Supplemental Table 1. RSD= Residual Standard Deviation; for abbreviations see Table 1 in main text.

Results

Supplemental Table 2 shows the differential count of monocytes, basophils and lymphocytes and baseline pre-and post-bronchodilator physiological values and number and percentage of asthmatics with abnormal physiologic values (>ULN or <LLN).

Supplemental Table 2. Baseline physiological parameters pre- and post-bronchodilator and prevalence of abnormal physiological parameters based on LLN (Lower Limit of Normal) and upper Limit of normal (ULN, see methods)

Parameter	Asthma	Controls	P - value
Clinical characteristics			
WBC, 10^9 /L	6.4 (5.4 ; 7.8)	5.8 (4.9 ; 6.0)	<0.001
Monocytes, 10^9 /L	0.5 (0.4 ; 0.6)	0.4 (0.3 ; 0.5)	<0.001
Lymphocytes, 10^9 /L	1.9(1.6 ; 2.3)	1.8 (1.4 ; 2.2)	0.02
Basophils, 10^9 /L	0.0 (0.0 ; 0.1)	0.0 (0.0 ; 0.0)	0.003
FEV_1, L	2.64 (2.10 ; 3.31)	3.51 (3.04 ; 3.89)	<0.001

Post FEV_1, L	2.94 (2.34 ; 3.62)		
% Change FEV_1	9.19 (4.6 ; 16.7)		
FEV_1 < LLN, N (%)	305 (40.0)	2 (2.0)	<0.001
FEV_1/FVC, %	69 (60 ; 80)	80 (80 ; 80)	<0.001
Post FEV_1/FVC, %	74 (70 ; 80)		
FEV_1/FVC, < LLN, N (%)	389 (51.1)	2 (2.1)	<0.001
% Fall in FVC > ULN, N (%)	365 (73.1)	2 (2.0)	<0.001
FEF_{50}, L/sec	2.29 (1.51 ; 3.12)	3.70 (3.32 ; 4.28)	<0.001
Post FEF_{50}, L/sec	2.95 (2.01 ; 3.90)		
FEF_{50} < LLN, N (%)	375 (52.4)	3 (3.4)	<0.001
IVC, L	3.65 (2.90 ; 4.54)	4.31 (3.65 ; 5.17)	<0.001
Post IVC, L	3.82 (3.13 ; 4.79)		
IVC, L < LLN, N (%)	96 (15.6)	1 (1.2)	<0.001
FEF_{25-75}, L/sec	1.74 (1.09 ; 2.56)	3.07 (2.58 ; 4.19)	<0.001
Post FEF_{25-75}, L/sec	2.33 (1.51 ; 3.29)		
FEF_{25-75} < LLN, N (%)	489 (67.6)	15 (15.5)	<0.001
FEF_{25-75} < LLN, N (%)	374 (51.7)	4 (4.1)	<0.001
RV, L	2.15 (1.72 ; 2.65)	1.85 (1.53 ; 2.16)	<0.001
Post RV, L	2.00 (1.61 ; 2.41)		
RV > ULN , N (%)	210 (30.3)	11 (13.3)	0.001
TLC, L	6.03 (5.07 ; 7.16)	6.12 (5.40 ; 6.89)	0.42
Post TLC, L	5.96 (4.98 ; 7.12)		
TLC > ULN, N (%)	134 (18.1)	13 (13.4)	0.25
RV/TLC	0.35 (0.29 ; 0.42)	0.30 (0.25 ; 0.35)	<0.001
Post RV/TLC	0.33 (0.27 ; 0.38)		
RV/TLC > ULN , N (%)	163 (22.)	9 (9.3)	0.003
FRC, L	3.26 (2.64 ; 3.95)	3.22 (2.85 ; 3.62)	0.76
Post FRC , L	3.09 (2.52 ; 3.75)		
FRC > ULN, N (%)	157 (21.6)	13 (13.7)	0.07
Raw, kPa*s/L	0.33 (0.22 ; 0.53)	0.200 (0.16 ; 0.24)	<0.001
Post Raw, 1kPa*s/L	0.23 (0.16 ; 0.37)		
Raw > ULN, N (%)	146 (21.8)	6 (6.5)	<0.001
sGaw, 1/KPa*s	1.11 (0.80 ; 1.74)	1.53 (1.18 ; 2.12)	<0.001
Post sGaw, 1/KPa*s	1.51 (1.10 ; 2.24)		
sGaw < LLN, N (%)	8 (1.4)	0 (0.0)	0.25
R20, kPa/L/s	0.35 (0.30 ; 0.43)	0.30 (0.25 ; 0.36)	<0.001
Post R20, kPa/L/s	0.31 (0.26 ; 0.37)		
R20 > ULN, N (%)	145 (23.1)	9 (9.9)	0.004
R5-R20, kPa/L/s	0.07 (0.03 ; 0.16)	0.02 (0.00 ; 0.04)	<0.001
Post R5-R20, kPa/L/s	0.05 (0.02 ; 0.09)		
R5-R20 > ULN, N (%)	259 (42.4)	8 (8.9)	<0.001
X5, kPa/L/s	-0.13 (-0.20 ; -0.09)	-0.09 (-0.11 ; -0.08)	<0.001
Post X5, kPa/L/s	-0.11 (-0.15 ; -0.08)		
X5 < LLN, N (%)	182 (30.6)	4 (4.3)	<0.001
AX, kPa/L/s	0.62 (0.28 ; 1.77)	0.21 (0.14 ; 0.32)	<0.001
Post AX, kPa/L/s	0.32 (0.16 ; 0.75)		
AX > ULN, N (%)	217 (41.4)	6 (6.7)	<0.001

Scond*VT, L^{-1}	0.035 (0.02 ; 0.06)	0.015 (0.00 ; 0.03)	<0.001
Post Scond*VT, L^{-1}	0.028 (0.02 ; 0.04)		
Scond*VT, L^{-1}> ULN, N (%)	115 (30.1)	3 (4.0)	<0.001
Sacin*VT, L^{-1}	0.113 (0.08 ; 0.17)	0.088 (0.06 ; 0.13)	0.002
Post Sacin*VT, L^{-1}	0.103 (0.06 ; 0.15)		
Sacin *VT, L^{-1}> ULN, N (%)	73 (19.3)	4 (5.9)	0.007
CT Scan characteristics			
MLD Inspiratory, HU	-838 (-857; -812)	-840 (-854; -813)	0.65
MLD Expiratory, HU	-685 (66.9)	-662 (74.0)	0.04
Lung Volume Inspiratory, L	5.22 (4.37; 6.22)	5.45 (4.59; 6.75)	0.30
Lung Volume Expiratory, L	2.62 (2.14; 3.15)	2.54 (2.21; 3.08)	0.80
Lung Volume Ratio E/I	0.498 (0.426 ; 0.595)	0.467 (0.38 ; 0.56)	0.16
VI 856 Expiratory	7.82 (2.5; 19.5)	7.83 (1.5; 15.5)	0.35
VI 950 Inspiratory	3.64 (1.4 ; 7.8)	4.61(1.0 ; 8.5)	0.83
Percentile 15 Inspiratory	-921 (-935; -904)	-929 (-940 ; -899)	0.46
Median Wall Area Inspiratory	33.3 (28.5 ; 37.4)	35.4 (30.8 ; 39.8)	0.05
Median Total Area Inspiratory	52.3 (44.8 ; 60.5)	57.7 (50.5 ; 66.0)	0.01
Median %WA Inspiratory	63.0 (3.47)	61.7 (2.48)	0.003
Median WA/BSA Inspiratory	17.6 (3.57)	18.7 (3.28)	0.06
Pi10 Inspiratory	7.21 (6.59 ; 7.77)	6.70 (6.28 ; 7.84)	0.07
Po20 %WA Inspiratory	7.41 (6.67 ; 8.50)	7.33 (6.42 ; 9.02)	0.73

Legend to Supplemental Table 2. All parameters are presented as Mean (standard deviation), Median (Quartile1 - Quartile 3), or N (%) as appropriate. WBC=White Blood Cell, RV= Residual Volume, FRC=Functional Residual Capacity, , FEV_1=Forced Expiratory Volume in the 1st second, FVC=Forced Vital Capacity, FEF_{50}=Forced Expiratory Flow at 50% of Forced Vital Capacity, IVC=Inspiratory Vital Capacity, FEF_{25-75}=Forced Expiratory Flow at 25%-75% of Forced Vital Capacity, R5-R20=Peripheral Airway Resistance, X5=Resistance at 5 Hz, AX=Area of Reactance, PC_{20}=Provocative Concentration of methacholine causing a 20% fall in $FEV_1$from baseline, PD_{20}= Provocative Dose of methacholine causing a 20% fall in FEV_1 from baseline ,CT= Computed tomography, MLD Ratio E/I= Mean Lung Density Expiratory to Inspiratory ratio, E=Expiratory, I=Inspiratory, BSA= Body Surface Area (m^2), WA= Wall Area (mm^2), TA= Total Area (mm^2), VI=Voxel Index, Pi10= Internal perimeter of 10mm, Po20= Outer perimeter of 20mm.

Baseline measures were performed in all patients, apart from MBNW and CT scans that were performed in a subgroup (n=382 asthmatics with and n=391 without MBNW, and n=308 with and n= 465 without CT scan). Asthmatics with and without CT scan were quite similar in overall characteristics (Supplemental Table 3), with a significant difference between those with and without CT in FEV_1/FVC ratio (0.69 vs 0.70 respectively), reversibility (9.8% vs 8.7%) and IOS values, R5-R20 (0.09 vs 0.06 kPa/L/s), AX (0.81 vs 0.52 kPa/L/s) and X5 (-0.150 and -0.120 kPa/L/s). Those with CT scan also used more frequently ICS than those without CT available (43 vs 19%) with a higher mean daily BDP equivalent dose (711 vs 578 µg/day) and ICS/LABA (10 vs 20%).

Supplemental Table 3. Demographics and physiological characteristics of asthma participants with and without CT scans.

Parameter	Asthma Group with CT Scan	Asthma Group without CT Scan	P-value
Clinical SAD Score	-0.065 (-0.27 ; 0.30)	-0.147 (-0.29 ; 0.13)	0.002
Number of subject in Clinical SAD Group 1	167 (54.2)	285 (62.5)	
Number of subject in Clinical SAD Group 2	141 (45.8)	171 (37.5)	
Age, years	46 (34 ; 55)	46 (34 ; 54)	0.78
Gender, female	187 (60.7)	263 (56.6)	0.25
Race, Caucasian	270 (87.7)	410 (88.2)	0.06
Blood pressure Systolic, mmHg	123.5 (111 ; 131)	123 (115 ; 131)	0.43
Blood pressure Diastolic, mmHg	78 (70 ; 83)	80 (70 ; 85)	0.04
Heart rate, beats/min	72 (64 ; 79)	71 (65 ; 78)	0.86
Height, cm	167.55 (160.8 ; 175.7)	170 (161.0 ; 177.0)	0.13
Weight, kg	75.15 (64.6 ; 88.0)	76 (65.0 ; 89.0)	0.90
BMI, kg/m²	26.2 (23.4 ; 30.3)	26.04 (23.1 ; 29.6)	0.44
Atopy (phadiatop +)	213 (82.2)	241 (79)	0.34
FeNO (flow rate 50)	25 (17 ; 40)	24 (15 ; 37)	0.11
SABA	259 (84.1)	412 (88.6)	0.07
Short acting anticholinergics	4 (1.3)	5 (1.1)	0.75
LABA	40 (13)	46 (9.9)	0.18
ICS	105 (34.1)	79 (17)	<0.001
ICS daily dose (BDP-equivalent, mcg)	711.65 (474.6)	611.18 -401.05	0.13
Extra-fine ICS	43 (14)	15 (3.2)	<0.001
Non-extra-fine ICS	62 (20.1)	65 (14)	0.02
ICS/LABA	159 (51.6)	300 (64.5)	<0.001
ICS/LABA daily dose (BDP-equivalent, mcg)	829.85 (580.34)	912.92 -663.28	0.13
Extra-fine ICS/LABA	31 (10.1)	93 (20)	<0.001
Non-extra-fine ICS/LABA	128 (41.6)	208 (44.7)	0.38
Systemic corticosteroids	10 (3.2)	12 (2.6)	0.59
Systemic corticosteroids mean daily dose	10 (5.0 ; 20.0)	6.25 (5.0 ; 20.0)	0.84
Montelukast	52 (16.9)	92 (19.8)	0.31
Lama	15 (4.9)	14 (3)	0.18
Biologics	7 (2.3)	25 (5.4)	0.03
GINA Step 1	47 (15.3)	88 (18.9)	0.07
GINA Step 2	45 (14.6)	40 (8.6)	0.07
GINA Step 3	79 (25.6)	128 (27.5)	0.07
GINA Step 4	122 (39.6)	178 (38.3)	0.07
GINA Step 5	15 (4.9)	31 (6.7)	0.07
Smoker, Current Smoker, n (%)	10 (3.2)	17 (3.7)	0.95
Smoker, Ex-smoker	63 (20.5)	93 (20)	0.95
Number of Pack-Years	4 (1.9 ; 7.5)	4.5 (2.0 ; 7.5)	0.52
Duration Smoking, years	12 (5.9 ; 18.1)	11.5 (7.0 ; 20.0)	0.65
Neutrophils, 10^9/L	3.745 (2.97 ; 4.66)	3.625 (2.95 ; 4.74)	0.75
Eosinophils, 10^9/L	0.25 (0.13 ; 0.39)	0.21 (0.13 ; 0.37)	0.31
PC_{20} and PD_{20} category, Moderate Severe	130 (52.8)	141 (44.9)	0.003
PC_{20}, mg/mL	1.06 (0.4 ; 3.0)	1.47 (0.5 ; 5.9)	0.06

PD_{20}, mg/mL	0.04 (0.0 ; 0.4)	0.2 (0.1 ; 0.7)	<0.001
Fall in FVC, %	17 (12.0 ; 23.0)	17 (11.0 ; 22.0)	0.44
Fall in FVC, % predicted	351.74 (231.8 ; 629.4)	363.99 (226.3 ; 548.4)	0.39
Fall in FVC > LLN	148 (74.7)	217 (72.1)	0.51
FEV_1, L	2.645 (2.00 ; 3.25)	2.64 (2.13 ; 3.42)	0.20
FEV_1, %predicted	82.6 (68.6 ; 93.8)	83.0 (71.0 ; 93.7)	0.52
FEV_1 < LLN	130 (42.2)	175 (38.5)	0.30
Change FEV_1, %predicted	8.1 (4.5 ; 13.1)	7.1 (3.9 ; 12.2)	0.04
% Change FEV_1	9.83 (5.3 ; 17.2)	8.67 (4.1 ; 16.3)	0.04
FEV_1/FVC, L	0.69 (0.6 ; 0.8)	0.7 (0.6 ; 0.8)	0.03
FEV_1/FVC, %predicted	84.2 (75.7 ; 92.0)	86.9 (77.3 ; 95.2)	0.007
FEV_1/FVC < LLN	175 (57)	214 (47.1)	0.008
FEF_{50}, L/sec	2.185 (1.45 ; 3.06)	2.39 (1.54 ; 3.28)	0.07
FEF_{50}, %predicted	60.0 (40.5 ; 80.3)	64.1 (44.9 ; 85.3)	0.03
FEF_{50} < LLN	164 (56.6)	211 (49.5)	0.07
FEF_{25-75}, L/sec	1.7 (1.06 ; 2.66)	1.76 (1.11 ; 2.52)	0.70
FEF_{25-75}, %predicted	55.9 (36.6 ; 74.6)	56.6 (38.7 ; 76.3)	0.51
FEF_{25-75} < LLN	159 (51.6)	215 (51.8)	0.96
IVC, L	3.62 (2.85 ; 4.51)	3.65 (2.94 ; 4.56)	0.69
IVC, %predicted	99.4 (17.93)	98.6 (18.44)	0.56
IVC < ULN	43 (15.8)	53 (15.5)	0.92
RV, L	2.12 (1.69 ; 2.58)	2.2 (1.77 ; 2.70)	0.09
RV, %predicted	111.9 (95.4 ; 140.4)	119.6 (101.6 ; 138.6)	0.19
RV, L > LLN	84 (29.8)	126 (30.7)	0.81
TLC, L	5.95 (5.05 ; 7.02)	6.06 (5.09 ; 7.28)	0.30
TLC, %predicted	105.7 (95.9 ; 117.3)	104.4 (95.7 ; 114.1)	0.22
TLC > LLN	65 (22)	69 (15.6)	0.03
RV/TLC, ratio	0.344 (0.29 ; 0.43)	0.35 (0.29 ; 0.42)	0.98
RV/TLC, %predicted	104.7 (89.0 ; 126.6)	106.7 (93.9 ; 125.2)	0.49
RV/TLC > LLN	72 (24.3)	91 (20.6)	0.24
FRC, L	3.17 (2.62 ; 3.88)	3.28 (2.66 ; 3.98)	0.45
FRC, %predicted	108.5 (93.7 ; 127.6)	109.2 (93.2 ; 125.9)	0.68
FRC, L > LLN	66 (22.4)	91 (21)	0.63
Raw, kPa*s/L	0.32 (0.22 ; 0.47)	0.35 (0.23 ; 0.59)	0.03
Raw, %predicted	129.6 (90.4 ; 190.9)	151.9 (93.7 ; 263.8)	0.02
Raw > LLN	42 (16.4)	104 (25.1)	0.008
sGaw, 1/KPa*s	1.05 (0.79 ; 1.60)	1.16 (0.80 ; 1.87)	0.04
sGaw, %predicted	56.7 (41.7 ; 92.0)	62.3 (43.8 ; 99.1)	0.05
sGaw < ULN	6 (2.8)	2 (0.6)	0.06
R20, kPa/L/s	0.36 (0.30 ; 0.43)	0.35 (0.30 ; 0.42)	0.18
R20, %predicted	116.4 (97.4 ; 136.2)	113.7 (97.6 ; 133.2)	0.59
R20 > LLN	66 (24.5)	79 (21.9)	0.45
R5-R20, kPa/L/s	0.09 (0.03 ; 0.18)	0.06 (0.03 ; 0.14)	0.03
R5-R20, %predicted	344.0 (95.2 ; 701.4)	227.1 (90.8 ; 619.6)	0.08
R5-R20 > LLN	123 (47.1)	136 (38.9)	0.04
X5, kPa/L/s	-0.15 (-0.21 ; -0.10)	-0.12 (-0.18 ; -0.09)	<0.001
X5, %predicted	140.7 (101.7 ; 191.9)	126.4 (89.2 ; 179.3)	0.01
X5 < ULN	94 (37.2)	88 (25.7)	0.003

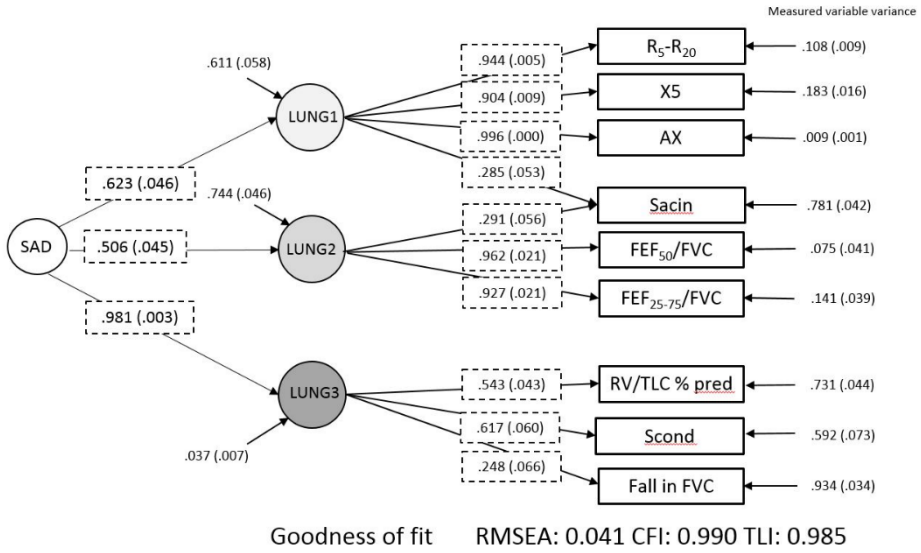
AX, kPa/L/s	0.81 (0.35 ; 1.90)	0.52 (0.23 ; 1.56)	0.002
AX, %predicted	247.8 (113.4 ; 607.3)	175.0 (77.6 ; 417.3)	<0.001
AX > LLN	103 (47)	114 (37.4)	0.03
Scond*VT, L^{-1}	0.036 (0.019 ; 0.053)	0.0334 (0.020 ; 0.058)	0.72
Scond*VT, %predicted	182.2 (102.8 ; 323.9)	175.3 (93.4 ; 302.3)	0.66
Scond*VT> LLN	56 (29.5)	59 (30.7)	0.79
Sacin*VT, L^{-1}	0.1083 (0.076 ; 0.162)	0.1183 (0.082 ; 0.170)	0.11
Sacin*VT, %predicted	106.3 (75.8 ; 144.6)	109.3 (79.6 ; 158.4)	0.28
Sacin *VT> LLN	36 (19)	37 (19.5)	0.92

Legend to Supplemental Table 3. For abbreviations see Supplemental Table 2.

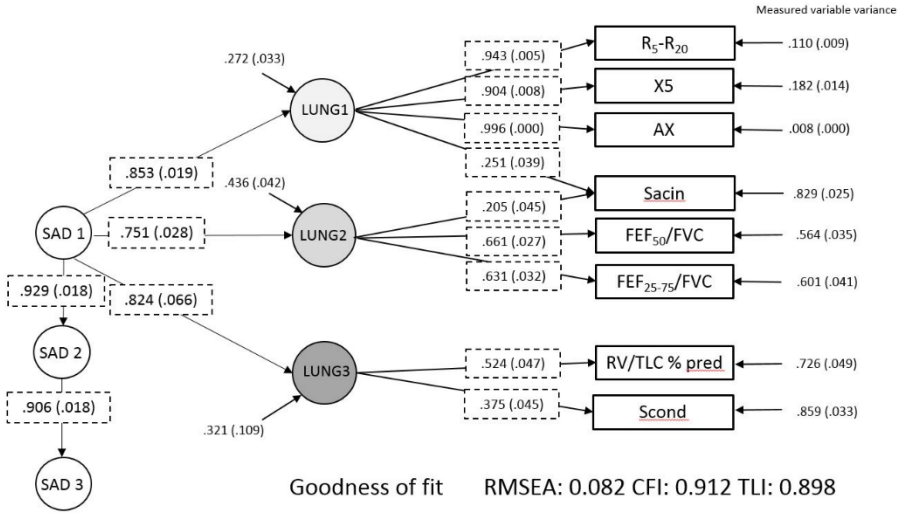
SEM models

Supplemental Figure 2A presents the same model including hyperresponsiveness testing as well. The % Fall in FVC during hyperresponsiveness testing loaded to the third latent variable, without much change in the RMSEA, CFI and TLI parameters and the correlation between the baseline model without and with Fall in FVC highly correlated (r=0.99). Figure 2B presents the Clinical SAD model without Fall in FVC that was tested at Visit 2 and Visit 3, and the same model structure was confirmed at the three visits. Figure 2C presents the CT SEM analyses of small airway function.

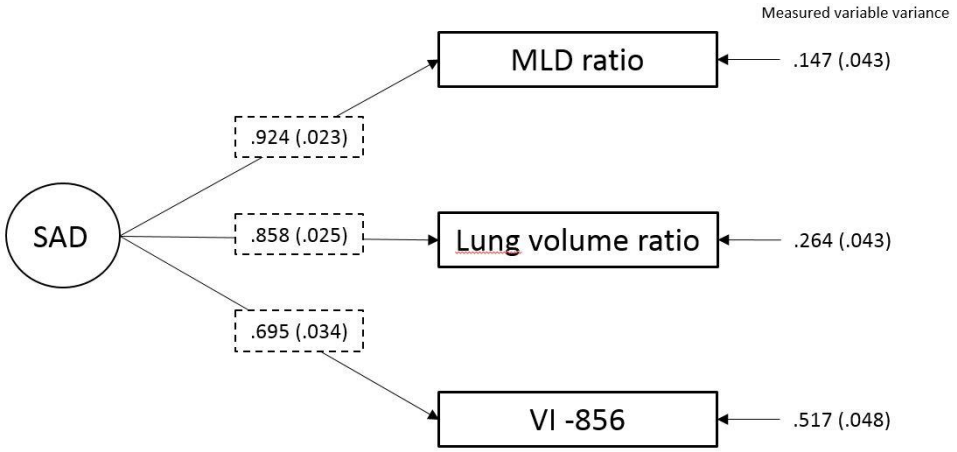
Supplemental Figure 2A. Cross-Sectional Clinical SEM analysis of small airway function with fall in FVC during hyperresponsiveness testing



Supplemental Figure 2B. Longitudinal Clinical SEM analyses of small airway function



Supplemental Figure 2C. CT SEM analyses of small airway function



Legend to Supplemental Figure 2. RMSEA= Root Mean Square Error of Approximation, CFI= Comparative Fit Index, TLI= Tucker-Lewis Index. For the other abbreviations, see supplemental Table 2. Figure 2A shows the model of clinical SEM analysis based on small airways parameters that were available at baseline. Figure 2B shows the model of clinical SEM analysis based on three visits of the longitudinal part of Atlantis. SAD1, SAD2 and SAD3, represent the assessments of SEM analysis at visits 1 (baseline), visit 2, after 6 months, and visit 3 after 12 months of follow-up respectively. There is a high correlation between the three models. Figure 2C shows the model of clinical SEM analysis based CT parameters

Supplemental Table 4 presents the predicted, >ULN and < LLN values of physiologic parameters for Clinical SAD Group1 and Clinical SAD Group2.

Supplemental Table 4. Physiological parameters for the two Clinical SAD Groups including predicted values, >ULN and < LLN ranges

Parameter	**Group 1**	**Group 2**	**P - value**
Clinical characteristics			

WBC, 10^9 /L	6.1 (5.4;7.5)	6.9 (5.8;8.1)	<0.001
Monocytes, 10^9 /L	0.44 (0.37 ; 0.55)	0.50 (0.39 ; 0.60)	0.003
Lymphocytes, 10^9 /L	1.82 (1.53 ; 2.18)	1.94 (1.60 ; 2.33)	0.01
Basophils, 10^9 /L	0.03 (0.02 ; 0.05)	0.04 (0.02 ; 0.06)	0.02
PC_{20}, mg/mL	1.5 (0.5 ; 4.8)	0.91 (0.2 ; 4.0)	0.006
PD_{20}, mg	0.23 (0.1 ; 0.8)	0.04 (0.0 ; 0.2)	<0.001
PC_{20} and PD_{20} categories			
Very mild, N (%)	78 (22)	40 (20)	<0.001
Mild, N (%)	102 (29)	33 (16)	<0.001
Moderate-severe, N (%)	146 (41)	123 (61)	<0.001
Fall in FVC, %	17.0 (11.0 ; 22.0)	18.5 (14.0 ; 23.0)	0.02
Fall in FVC > ULN, N (%)	238 (70)	126 (81)	0.01
FEV_1, L	2.96 (2.50 ; 3.61)	2.105 (1.68 ; 2.65)	<0.001
Post FEV_1, L	3.23 (2.72 ; 3.89)	2.42 (1.97 ; 3.05)	<0.001
% Change FEV_1	7.02 (3.9 ; 11.8)	15.17 (7.3 ; 24.2)	<0.001
FEV_1 < LLN, N (%)	94 (21)	211 (68)	<0.001
FEV_1/FVC, %	73 (70 ; 80)	63 (60 ; 70)	<0.001
Post FEV_1/FVC, %	78 (70 ; 80)	68 (60 ; 70)	<0.001
FEV_1/FVC, L < LLN, N (%)	156 (35)	233 (75)	<0.001
FEF_{50}, L/sec	2.79 (2.13 ; 3.61)	1.53 (1.05 ; 2.13)	<0.001
Post FEF_{50}, L/sec	3.48 (2.76 ; 4.26)	2.06 (1.51 ; 2.89)	<0.001
FEF_{50}< LLN, N (%)	143 (34)	232 (79)	<0.001
IVC, L	4.02 (3.27 ; 4.83)	3.26 (2.55 ; 4.05)	<0.001
Post IVC, L	4.19 (3.38 ; 4.94)	3.52 (2.81 ; 4.43)	<0.001
IVC < LLN, N (%)	40 (11)	56 (0.213)	<0.001
FEF_{25-75}, L/sec	2.28 (1.65 ; 2.98)	1.11 (0.77 ; 1.58)	<0.001
Post FEF_{25-75}, L/sec	2.92 (2.12 ; 3.71)	1.55 (1.08 ; 2.25)	<0.001
FEF_{25-75} < LLN, N (%)	140 (33)	234 (0.775)	<0.001
RV, L	1.99 (1.62 ; 2.40)	2.475 (1.99 ; 3.06)	<0.001
Post RV, L	1.95 (1.54 ; 2.26)	2.1 (1.72 ; 2.61)	<0.001
RV > ULN, N (%)	71 (17)	139 (50)	<0.001
TLC, L	6.080 (5.21 ; 7.10)	5.900 (4.88 ; 7.19)	0.08
Post TLC, L	6.040 (5.20 ; 7.16)	5.780 (4.85 ; 7.08)	0.02
TLC > ULN, N (%)	72 (16.3)	62 (20.8)	0.12
RV/TLC	0.31 (0.27 ; 0.37)	0.42 (0.34 ; 0.48)	<0.001
Post RV/TLC	0.31 (0.26 ; 0.36)	0.36 (0.30 ; 0.42)	<0.001
RV/TLC >ULN, N (%)	44 (10)	119 (40)	<0.001
FRC, L	3.19 (2.64 ; 3.85)	3.325 (2.67 ; 4.01)	0.23
Post FRC, L	3.145 (2.59 ; 3.76)	2.94 (2.42 ; 3.68)	0.06
FRC Pleth > ULN, N (%)	79 (18.1)	78 (26.7)	0.04
Raw, kPa*s/L	0.27 (0.20 ; 0.41)	0.46 (0.32 ; 0.69)	<0.001
Post Raw, kPa*s/L	0.2 (0.15 ; 0.29)	0.3 (0.20 ; 0.46)	<0.001
Raw > ULN, N (%)	21 (15.1)	18 (17.0)	0.70
sGaw, 1/KPa*s	1.21 (0.87 ; 1.89)	0.89 (0.65 ; 1.40)	<0.001
Post sGaw, 1/KPa*s	1.65 (1.23 ; 2.48)	1.285 (0.88 ; 1.88)	<0.001
sGaw, (1/kPa*s) < LLN, N (%)	4 (1.0)	4 (2.2)	0.27
R20, kPa/L/s	0.33 (0.27 ; 0.39)	0.4 (0.34 ; 0.47)	<0.001
Post R20, kPa/L/s	0.29 (0.24 ; 0.34)	0.35 (0.30 ; 0.43)	<0.001

R20 > ULN, N (%)	59 (16)	86 (0.347)	<0.001
R5-R20, kPa/L/s	0.04 (0.01 ; 0.07)	0.18 (0.12 ; 0.27)	<0.001
Post R5-R20, kPa/L/s	0.03 (0.01 ; 0.06)	0.1 (0.05 ; 0.18)	<0.001
R5-20 > ULN, N (%)	57 (15)	202 (86)	<0.001
X5, kPa/L/s	-0.11 (-0.14 ; -0.08)	-0.22 (-0.28 ; -0.17)	<0.001
Post X5, kPa/L/s	-0.09 (-0.11 ; -0.07)	-0.15 (-0.21 ; -0.12)	<0.001
X5 < LLN, N (%)	28 (7)	154 (72)	<0.001
AX, kPa/L/s	0.33 (0.19 ; 0.60)	2.14 (1.49 ; 3.16)	<0.001
Post AX, kPa/L/s	0.22 (0.13 ; 0.34)	0.83 (0.43 ; 1.61)	<0.001
AX > ULN, N (%)	37 (12)	180 (90)	<0.001
Scond*VT, L^{-1}	0.0267 (0.015 ; 0.042)	0.0491 (0.038 ; 0.075)	<0.001
Post Scond*VT, L^{-1}	0.0217 (0.013 ; 0.035)	0.0397 (0.024 ; 0.058)	<0.001
Scond*VT> ULN, N (%)	49 (20)	66 (49)	<0.001
Sacin*VT, L^{-1}	0.0943 (0.070 ; 0.134)	0.1533 (0.114 ; 0.197)	<0.001
Post Sacin*VT, L^{-1}	0.0841 (0.059 ; 0.138)	0.1273 (0.091 ; 0.176)	<0.001
Sacin *VT> ULN, N (%)	30 (12)	43 (32)	<0.001

Legend to Supplemental Table 4. For Abbreviations see supplemental Table 2.

Correlations of CT SAD score with physiologic and clinical parameters

Supplemental Figure 3 shows the correlations between the CT SAD score, based on the CT SEM model, and parameters measured. Correlations were much lower than those of the clinical SAD score that showed r-values >0.6. The highest correlations of the CT SAD score (>0.4 and <-0.4) existed for duration of smoking, age and RV/TLC (positively) and FEV_1 (negatively). The CT SAD score did not significantly associate with ACT (r=-0.05, p=0.367), but did so modestly with the number of exacerbations (r=+0.12, p=0.04). The CT SAD score was significanlty different across GINA severities, mean SAD score in GINA 1 being -0.747, GINA 2 – 0.459, GINA 3 -0.408, GINA 4 -0.166 and GINA 5 +0.088 (ANOVA p=0.013).

Model-based clustering defining CT SAD Groups

Two groups, n=169 and n=125, were identified based on the CT SAD parameters within participants who underwent CT scanning (clinical characteristics: see Supplemental Tables 5

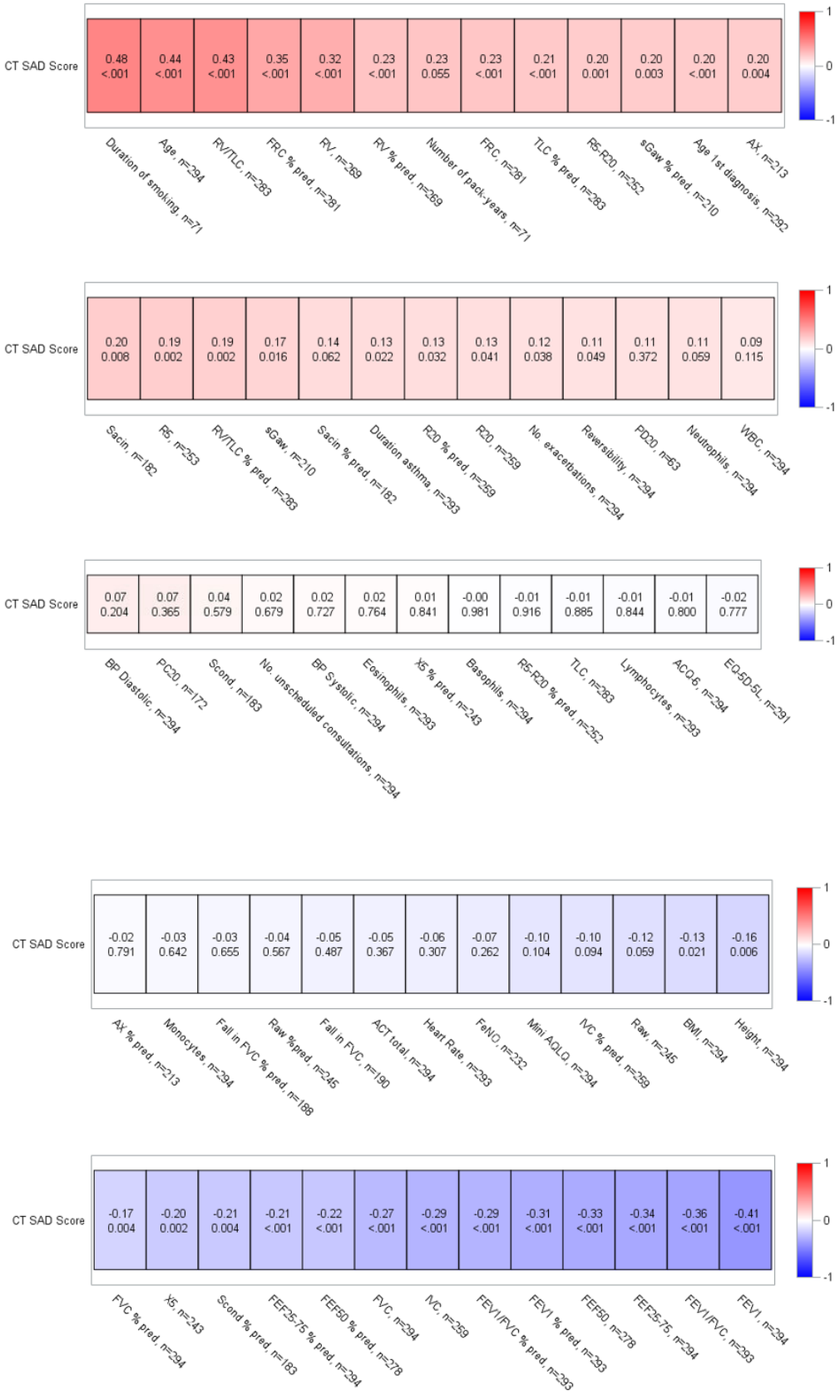
and 6). The two CT SAD groups demonstrated significantly different clinical SAD scores and CT SAD scores and were comparable in heart rate, blood pressures, height, weight, atopy, FeNO levels, smoking habits, blood cell differential counts, ACT score and duration of disease. They used comparable asthma drugs, apart from LABA and montelukast use being higher in Group2 vs Group1 (17 vs 9%, 25 vs12%). Group1 was significantly younger, and demonstrated, higher BMI, female prevalence and GINA stage. Sacin values were comparable between Group1 and controls, while Sacin was significantly different between Group2 and controls. Group1 and 2 had comparable values of hyperresponsiveness (severity and fall in FVC), Scond, R5-R20, Ax and X5 values. Overall, Group1 had otherwise fewer abnormal physiologic parameters. As expected, the small airway parameters of the CT-scan were significantly different between the two Groups; inspiratory parameters were comparable.

Clinical SAD analysis in patients with CT scans available

Because CT scans were available in 294 out of 739 asthma patients, we performed additional SEM analysis on clinical SAD parameters in this subset. The model structure and parameters estimates were very similar to the overall model. Including CT scan variables into the model (Supplemental Figure 2C) provided an additional latent variable (the CT scan). The weight of this latent variable in the overall measure of SAD was 0.321 (factor loading) and r^2 was 0.1, showing that the low relation between CT-scan variables and non-CT clinical variables.

Supplemental Figure 3 presents the correlations of all parameters measured and the CT SAD score.

Supplemental Figure 3. CT SAD score: correlation with all parameters measured



Legend to Supplemental Figure 3. For abbreviations see Supplemental Table 1.

Supplemental Table 5. Clinical characteristics of asthma patients in CT SAD Group1 and CT SAD Group2

Parameter	Group1 (n=169)	Group2 (n=125)	P-value
Clinical SAD score	-0.12 (-0.28; 0.21)	-0.005 (-0.24 ; 0.40)	0.04
CT Scan SAD score	-1.01 (-0.74)	0.54 (-0.76)	<0.001
Age,years	43 (31 ; 54)	50 (40 ; 56)	0.002
Gender, female N (%)	96 (57)	82 (66	0.13
Heart rate, bpm	72.0 (65 ; 80)	70.0 (64 ; 77)	0.20
BP syst, mmHg	124 (114 ; 131)	121 (110 ; 130)	0.25
BP diast, mmHg	77 (70 ; 83)	79 (70 ; 83)	0.99
BMI, kg/m^2	27 (24 ; 30)	25(22 ; 29)	0.008
Atopy, N (%)	114 (81)	89 (83)	0.72
FeNO, L/min	28 (17 ; 43)	24 (17 ; 37)	0.37
Ex-smoking, N (%)	33 (20)	29 (23)	0.73
Duration smoking, years	10.0 (5.0 ; 16.0)	12.2 (9.0 ; 19.0)	0.15
GINA 1/2, N (%)	61 (37)	29(23)	0.007
GINA 3, N (%)	50 (30)	27 (22)	0.007
GINA 4/5, N (%)	58 (34)	69 (55)	0.007
ICS uncombined, N(%)	52 (31)	47 (38)	0.08
ICS/LABA, N(%)	84 (50)	68 (54)	0.43
ICS dose, BDP equivalent, µg	627.5(415.8)	802.2(541.9)	0.10
ICS/LABA dose, BDP equivalent, µg	724.8 (418.4)	962.1 (723.5)	0.05
Oral corticosteroids, N (%)	3 (1.8)	7 (5.6)	0.10
WBC, 10^9 /L	6.6 (5.4 ; 7.7)	6.5 (5.6 ; 7.7)	0.78
Eosinophils, 10^9 /L	0.25 (0.15 ; 0.38)	0.24 (0.13 ; 0.40)	0.10
Neutrophils, 10^9 /L	3.72 (2.90 ; 4.52)	3.67 (3.00 ; 4.59)	0.67
Monocytes, 10^9 /L	0.46 (0.39 ; 0.59)	0.46 (0.38 ; 0.56)	0.84
Lymphocytes, 10^9 /L	1.90 (1.51 ; 2.24)	1.85 (1.50 ; 2.30)	0.74
Basophils, 10^9 /L	0.03 (0.02 ; 0.06)	0.03 (0.02 ; 0.06)	0.47
PC_{20}, mg/mL	1.14 (0.4 ; 3.3)	0.98 (0.3 ; 3.0)	0.55
PD_{20}, mg	0.03 (0.0 ; 0.3)	0.09 (0.0 ; 0.6)	0.09
PC_{20} and PD_{20} categories			
Very mild, N (%)	23 (16.2)	20 (21.5)	0.74
Mild, N (%)	37 (26.1)	25 (26.9)	0.74
Moderate-severe, N (%)	78 (54.9)	46 (49.5)	0.74
Fall in FVC, %	17.0 (13.0 ; 21.0)	17.00 (11.0 ; 23.5)	0.78
FEV_1, L	2.86 (2.21 ; 3.45)	2.27 (1.80 ; 2.91)	<0.001
Post FEV_1, L	3.15 (2.45 ; 3.95)	2.69 (2.04 ; 3.21)	<0.001
% Change FEV_1	9.06 (5.1 ; 16.7)	11.11 (5.4 ; 19.7)	0.24
FEV_1/FVC, %	71 (60 ; 80)	65 (60 ; 70)	<0.001
Post FEV_1/FVC, %	76 (70 ; 80)	70 (60 ; 80)	<0.001
FEF_{50}, L/sec	2.36 (1.77 ; 3.25)	1.84 (1.09 ; 2.90)	<0.001
Post FEF_{50}, L/sec	3.23 (2.23 ; 4.09)	2.36 (1.59 ; 3.50)	<0.001
IVC, L	3.82 (3.12 ; 4.71)	3.39 (2.66 ; 4.27)	0.002
Post IVC, L	4.10 (3.24 ; 5.02)	3.59 (2.83 ; 4.41)	0.001
FEF_{25-75}, L/sec	1.92 (1.32 ; 2.81)	1.38 (0.78 ; 2.32)	<0.001

Post FEF_{2575}, L/sec	2.64 (1.68 ; 3.53)	1.82 (1.10 ; 2.92)	<0.001
RV, L	1.95 (1.60 ; 2.47)	2.17 (1.85 ; 2.73)	0.002
Post RV, L	1.79 (1.44 ; 2.13)	2.06 (1.66 ; 2.44)	<0.001
TLC, L	6.040 (5.04 ; 7.05)	5.86 (5.01 ; 6.99)	0.68
Post TLC, L	5.99 (4.96 ; 7.30)	5.81 (4.96 ; 6.83)	0.45
RV/TLC, L	0.32 (0.26 ; 0.40)	0.38 (0.32 ; 0.48)	<0.001
Post RV/TLC, L	0.26 (0.24 ; 0.34)	0.35 (0.29 ; 0.41)	<0.001
FRC, L	3.01 (2.53 ; 3.74)	3.37 (2.82 ; 4.00)	0.009
Post FRC, L	2.90 (2.31 ; 3.57)	3.20 (2.67 ; 3.76)	0.005
Raw, kPa*s/L	0.29 (0.21 ; 0.46)	0.33 (0.22 ; 0.47)	0.32
Post Raw, kPa*s/L	0.20 (0.15 ; 0.28)	0.21 (0.16 ; 0.32)	0.46
sGaw, 1/KPa*s	1.07 (0.80 ; 1.60)	1.04 (0.76 ; 1.54)	0.84
Post sGaw, 1/KPa*s	1.59 (1.21 ; 2.10)	1.48 (1.11 ; 2.07)	0.82
R20, kPa/L/s	0.35 (0.31 ; 0.42)	0.37 (0.30 ; 0.46)	0.27
Post R20, kPa/L/s	0.31 (0.27 ; 0.36)	0.33 (0.27 ; 0.41)	0.07
R5-R20, kPa/L/s	0.08 (0.03 ; 0.16)	0.10 (0.03 ; 0.18)	0.31
Post R5-R20, kPa/L/s	0.04 (0.02 ; 0.09)	0.06 (0.02 ; 0.12)	0.07
X5, kPa/L/s	-0.14 (-0.21 ; -0.10)	-0.15 (-0.21 ; -0.11)	0.14
Post X5, kPa/L/s	-0.10 (-0.15 ; -0.07)	-0.11 (-0.17 ; -0.09)	0.04
AX, kPa/L/s	0.73 (0.31 ; 1.83)	0.92 (0.44 ; 1.95)	0.23
Post AX, kPa/L/s	0.32 (0.16 ; 0.87)	0.38 (0.22 ; 0.83)	0.17
Scond*VT, L^{-1}	0.04 (0.02 ; 0.05)	0.04 (0.020 ; 0.054)	0.71
Post Scond*VT, L^{-1}	0.02 (0.01 ; 0.04)	0.03 (0.014 ; 0.051)	0.06
Sacin*VT, L^{-1}	0.10 (0.07 ; 0.13)	0.13 (0.095 ; 0.191)	0.003
Post Sacin*VT, L^{-1}	0.082 (0.06 ; 0.13)	0.12 (0.082 ; 0.171)	0.001
Unscheduled consultations, N	0.27	0.38	0.80
No. exacerbations	0.18	0.34	0.12
> 1 exacerbation, N (%)	22 (13)	24 (19)	0.03
Duration of disease, years	19.74 (7.6 ; 29.7)	21.56 (8.0 ; 35.8)	0.12
Age at 1st Diagnosis	19	20	0.75
Age at 1st Diagnosis < 18 years, N (%)	79 (47)	58 (46)	0.88
ACT, total score	21 (18 ; 24)	21 (18 ; 23)	0.13
ACT score < 15, N (%)	24 (14.2)	19 (15.2)	<0.001
ACQ-6, total mean score	0.67 (0.3 ; 1.7)	0.83 (0.3 ; 1.3)	0.63
ACQ-6 score > 1.25, N (%)	60 (35.5)	38 (30.4)	<0.001
EQ-5D-5L: VAS score	80 (70.0 ; 90.0)	80 (70.0 ; 90.0)	0.48

Legend to Supplemental Table 5. Data are presented as %, Mean (SD) and Median (interquartile ranges) as appropriate; for abbreviations see Supplemental Table 1 .

Supplemental Table 5 presents the predicted, >ULN and < LLN values of physiologic parameters for CT SAD Group1 and CT SAD Group2.

Supplemental Table 6. physiological parameters for CT SAD Group1 and Group2, including predicted values, ULN and LLN

Parameter	Group 1 (n=169)	Group 2 (n=125)	P - value
FEV_1, %predicted	85.99 (73.0 ; 96.5)	75.26 (61.8 ; 90.4)	<0.001
Change FEV_1, %predicted	7.93 (4.6 ; 13.2)	8.18 (4.3 ; 13.0)	0.10
FEV_1/FVC, %predicted	86.14 (79.7 ; 92.1)	80.45 (69.9 ; 90.0)	<0.001
FEV_1 < LLN, N (%)	56 (33.1)	65 (52.0)	0.001
FEV_1/FVC, L < LLN , N (%)	86 (50.9)	80 (64.5)	0.02
FEF_{50}, %predicted	62.83 (48.2 ; 84.6)	53.16 (31.9 ; 78.8)	0.001
FEF_{50} < LLN, N (%)	84 (52.2)	71 (60.7)	0.16
IVC, %predicted	101.32 (17.13)	97.71 (18.67)	0.11
IVC < LLN, N (%)	22 (15.1)	18 (15.9)	0.85
FEF_{25-75}, %predicted	50.34 (37.0 ; 69.1)	40.77 (22.1 ; 62.1)	<0.001
FEF_{25-75} < LLN , N (%)	108 (63.9)	91 (72.8)	0.11
FEF_{25-75}, %predicted	59.01 (44.3 ; 76.1)	48.63 (26.7 ; 68.8)	<0.001
FEF_{25-75} < LLN, N (%)	76 (45.0)	75 (60.0)	0.01
RV, %predicted	107.14 (90.9 ; 129.5)	125.12 (102.0 ; 148.5)	<0.001
RV > ULN, N (%)	36 (22.8)	43 (38.7)	0.005
TLC, %predicted	104.57 (95.9 ; 115.6)	107.69 (96.1 ; 120.0)	0.09
TLC > ULN, N (%)	30 (18.2)	34 (28.8)	0.04
RV/TLC %predicted	97.71 (82.8;115.7)	109.90 (93.6 ; 133.8)	<0.001
RV/TLC > ULN, N (%)	30 (18.2)	37 (31.4)	0.01
FRC, %predicted	103.80 (90.2 ; 119.9)	113.85 (100.7 ; 132.3)	<0.001
FRC > ULN, N (%)	30 (18.2)	33 (28.4)	0.04
Raw, %predicted	123.84 (86.3 ; 187.7)	137.71 (97.0 ; 211.6)	0.21
Raw > ULN, N (%)	21 (15.1)	18 (17.0)	0.69
sGaw, %predicted	60.09 (42.5 ; 91.6)	55.72 (41.3 ; 93.2)	0.69
sGaw < LLN, N (%)	3 (2.4)	2 (2.4)	1.000
R20, %predicted	113.20 (98.4 ; 131.3)	122.31 (94.5 ; 140.8)	0.30
R20 > ULN, N (%)	28 (19.0)	36 (32.1)	0.02
R5-R20, %predicted	320.84 (125.0 ; 728.2)	366.85 (51.2 ; 651.3)	0.94
R5-R20 > ULN, N (%)	61 (42.7)	57 (52.3)	0.13
X5, %predicted	141.06 (101.7 ; 190.5)	140.23 (101.5 ; 188.4)	0.75
X5 < LLN, N (%)	52 (36.6)	36 (35.6)	0.88
AX, %predicted	242.26 (104.5 ; 616.7)	250.06 (132.1 ; 579.6)	0.75
AX > ULN, N (%)	55 (44.4)	45 (50.6)	0.37
% fall in FVC > ULN, N (%)	90 (78.9)	53 (69.7)	0.15
Scond*VT, %predicted	182.35 (110.6 ; 340.8)	178.72 (97.3 ; 277.5)	0.38
Scond*VT> ULN, N (%)	34 (31.2)	19 (25.7)	0.42
Sacin*VT, %predicted	93.53 (72.1 ; 129.3)	122.63 (84.3 ; 175.8)	0.007
Sacin *VT, L^{-1}> ULN, N (%)	14 (12.8)	21 (28.8)	0.008

Legend to Supplemental Table 6. For abbreviations see Supplemental Table 1.

Supplemental Table 7. Comparison of ATLANTIS clinical characteristics with data from the literature

Parameter	**Atlantis N=773**	**Ref 15 2015 N=442**	**Ref 16 2012 N=378**	**Ref 17 2013 All n=441**	**Ref 17 2013 With PAO N=219**	**Ref 17 2013 WO PAO N=222**	**Ref 18 2014 N=66 Sacin> 0.12***	**Ref 19 2013 N=33**	**Ref 20 2014 N=43/ 31 M-MS/ Sev***	**Ref 21 2016 n=169**
Age, years	46	42	42	-	-	44	53	45	57/54	44
Female %	58	64	63	-	-	57	50	69	49/61	61
BMI, kg/m^2	26	-	28	-	-	26	-	24	27/30	26
Atopy, %	81	-	-	-	-	73	-	66	79/71	60
Ex-smoker, %	20	?		-	-	15	-	-	?	31
Curr smoker,%	4	0	10	-	-	13	-	18	0	4
ICS, % (dose)	84 (651)	94 (800)	100 (± 800)	100 (-)	100 (-)	100 (-)	100 (≤800)	60 (500-1000)	547/17 26	85(-)
EF ICS, %	7.5	-	9	<5%	<5%	-	-	-	-	-
ICS/LABA %	54	-	-	100	100	100	-	54	-	-
Exacerbation , N	0.21	-	-	-	-	1.2	-	-	1.0/3.5	-
Emergency vis, N	0.1	-	-	-	-	0.1	-	-	-	-
ACT/ACQ6	21/0.8	-	-	-	20/-	20/-	23/-	22/-	1.0/1.8	21/0.8 (ACQ)
FEV_1, %pred	83	86	86	80	64	98	76	100	91/87	88
FEV_1/FVC,%	69	74		74	68	81	66	-	73/68	73
MEF_{50},%pred	62	-	-	-	-	-	-	-	-	62
FEF_{25-75}, %pred	57	60	60	60	43	78	44	62	-	-
FEF_{25-75} <LLN	68			26	-	-	-	-	-	-
FEF_{25-75} <60%pred, %	57	54	±50	-	-	-	-	-	-	-
FEV_1%>80 and FEV_1/FVC>0.7, %	27	-	-	-	-	12	-	62	-	-
FEV_1%≤80 and FEV_1/FVC≤0.7, %	96	-	-	-	41	-	-	-	-	-
R20, kPa/L/s	0.35	-	-	-	-	-	-	0.31	0.31/0. 39	0.37
R20, %pred	114	-	133 132 137	-	-	-	-	-	-	-
R20>ULN	23.1	-	-	-	-	-	-	-	-	30
R5-R20, kPa/L/s	0.07	0.07	0.09 0.08 0.12	-	-	-	0.06	0.06	0.05/0. 05	0.09
R5-R20>ULN, %	42	-	-	-	-	-	-	-	-	31
R5-R20 >0.10, %	37	43	-	-	-	-	-	-	-	-
R5-R20 =0.03, %	70	-	65 64 70	-	-	-	-	-	-	-
R5-R20>0.075, %	33	-	-	-	-	-	-	66	-	-
AX, kPa/L/s	0.62	-	-	-	-	-	-	-	0.41/0. 47	0.34
AX>LLN, %	41	-	-	-	-	-	-	-	-	33
X5, kPa/L/s	-0.13	-	-	-	-	-	-0.10	-0.11	-0.13/- 0.15	-
X5<LLN, %	31	-	-	-	-	-	-	-	-	-
FRC, %pred	109	-	-	-	120	109	-	-	-	-
FRC>LLN, %	22	-	-	-						

FRC>120%pred	16	-	-	-	37	26	-	-	-	-
RV, %pred	117	-	-	-	133	122	-	-	-	-
RV>LLN, %	30	-	-	-	43	31	-	-	-	-
FEV_1<60%pred, %	66	-	-	-			-	-	-	-
$FEV_1 \leq$80%pr and $FEV_1/FVC \leq 0.7$, %	51	-	-	-	44		-	-	-	-
FEV_1>80%pr and FEV_1/FVC>0.7, %	15	-	-	-		31	-	-	-	-
RV/TLC,%pred	106	-	-	-			-	-	110/109	-
RV/TLC>LLN, %	22	-	-	-	50	24	-	-	-	-
$FEV_1 \leq$80%pred and $FEV_1/FVC \leq 0.7$, %	43	-	-	-	50		-	-	-	-
FEV_1>80%pred and FEV_1/FVC>0.7, %	9	-	-	-	-	24	-	-	-	-
FEV_1>35%pred, %	49	-	-	-	-	-	52	-	-	-
FEV_1<60%pred	71	-	-	-	-	-		-	-	-
Scond*Vt, L^{-1}	0.035	-	-	-	-	-	?	-	0.051/0.038	0.027
Scond*VT,%pred	181	-	-	-	-	-	137	-	-	-
Scond*VT >LLN, %	30	-	-	-	-	-	-	-	-	47
Sacin*VT, L^{-1}	0.113	-	-	-	-	-	?	-	0.175/0.184	0.097
Sacin*VT, %pred	107	-	-	-	-	-	162	-	-	-
Sacin*VT >LLN, %	19	-	-	-	-	-	-	-	-	42
Sacin*VT >0.12	45	-	-	-	-	-	100	-	-	-

Legend to Supplemental Table 7. Comparison of the findings in ATLANTIS with those reported in the literature. *, inclusion criterion added , since this is not asthma-wide, but selected on Sacin (ref 18), or Moderate-severe asthma (ref 20) Ref number in top of Table refers to article results in Reference number in the Supplement. WO=Without, PAO= peripheral airway obstruction; M-MS= Mild to moderate severe asthma; Sev= severe asthma; Pred= predicted; EF=extra-fine; RSD= residual standard deviation; ICS dose = BDP equivalents per day, -= data not available in publication; Ref number 15 has British Thoracic Society data from BTS stage 2,3, and 4. When three numbers are presented they are from these BTS severity stages in that following order.

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Reviewer #4: Thank you for your responses and changes to the manuscript, and apologies for any conflicting recommendations with the journal. Thank you for clarifying my understanding of these parameters. I have only a few additional points to help clarify points in your manuscript.

Answer to Reviewer #4: *We thank the reviewer for these suggestions and have answered them point-by-point below.*

Minor comments:

1. Abstract: Findings: Sent 5: just say MBNW Sacin here, instead of defining it again after sent 2.

Answer to comment 1: *We have adjusted this as follows (crossed words were deleted):*

"Clinical-SAD Group1 (n=452) had "milder" SAD, i.e. comparable ~~ventilation heterogeneity in pre-acinar/acinar airways values (~~MBNW Sacin~~)~~ with controls. Group2 (n=312) had more abnormal physiologic SAD measures than Group1, particularly IOS and spirometry, and more severe asthma (asthma control, treatments, exacerbations, quality of life)."

2. Abstract: Interpretation: I wouldn't say these parameters contribute to SAD. For Sent 1, please try or edit "SAD is a complex feature of asthma, with multiple physiologic measures likely reflecting different disease components." I would drop the "further work is needed." I think it is, but I was thinking you would expand on the sentence and I agree there isn't room in the abstract.

Answer to comment 2: *We now have changed the abstract. However, we do not study asthma heterogeneity, but the complexity of small airway dysfunction (SAD) in asthma.* It is impossible to get a specific regional signal from the silent zone of the lungs unfortunately. *SAD* cannot be captured by one measurement. *Thus it was the main aim of the ATLANTIS study to assess many physiologic* measurements. Putting together all the available measurements we performed, we provide information about the relevant effect of small airway dysfunction (SAD) as assessed by IOS, MBNW, body plethysmography and spirometry. We have changed the sentence as follows (crossed words that were deleted and underlined *changes), and in addition followed the comment of the Editor (please see below for final result):*

"SAD is a complex and silent signature of asthma, which is likely to be directly or indirectly captured by combinations of physiologic tests: spirometry, body plethysmography, IOS, and MBNW. SAD is present across all asthma severity and particularly in severe disease. The clinical classification of SAD in two groups, i.e. a "milder" and "more severe" SAD group, by the easy-to-conduct measures IOS and spirometry, is meaningful given its association with GINA asthma severity stages, asthma control, quality of life, and exacerbations. ~~Further work is needed~~

3. Table 1. I think this table is great and adds a lot. I suggest adding a heading for column 2, such as "Interpretation".

Answer to comment 3: *We now have changed the Table according to suggestions in the Reviewer's Comment numbers 3-7 and added the abbreviations used in the Legend to the Table 1 see Table 1 at Answer to Comment 6 from reviewer #4*

4. Table 1. Would help to have a subheadings under physiologic (or instead of physiologic) for the clinical test the measure is from (i.e. spirometry, MBNW, IOS, methacholine challenge, etc.).

Answer to comment 4; *We have added these subheadings (please see Table below)*

5. Table 1. Could you add sGaw, as it is a parameter in table 4?

Answer to comment 5: *Table 4 shows the independent predictors of exacerbations in the Poisson Regression model and in the Supplement we wrote:*

"The relationships between lung physiology variables and healthcare consumption in asthmatics were analyzed using a Poisson regression model for each healthcare resource consumption variable. Only variables with a number of missing values approximately lower than 50% were considered in the analysis. The model included the healthcare resource consumption variable as dependent variable and all the lung physiology variables as independent variables. "

Here we thus included ALL lung physiology variables. We now have added this to the Legend of the Table as follows:

"All lung physiology parameters were included in the Poisson Regression model."

6. Table 1. I think the interpretations could be more clear (most of them seem to be for small-to-mid-sized airways), but perhaps these are just overlapping measures. Can you clarify whether X5 and R5-20 reflect the same level of airways (small-mid)? And AX overlaps with X5 (distensibility of small-mid sized airways)?

Answer to comment 6: *Thank you for pointing this out. Actually, measurements are overlapping as far as the regional location inside the respiratory system is concerned. However, each single measurement reflects different aspects or different functional effects of small-mid sized and / or peripheral airways physiology. For instance the mentioned parameters R5-R20, X5 and AX are all derived from the oscillation technique (IOS) that reflect SAD by different mechanism. Thus R5-R20 presents Respiratory System Resistance of small-to-mid-sized conductive and peripheral airways resistance, while X5 presents Respiratory System Reactance reflecting inertance and elasticity (capacitance) of the whole respiratory system including small peripheral airways; finally AX represents the distensibility of the peripheral lungs (parenchyma + small peripheral airways). Thus, though all show evidence of Small Airways Dysfunction, the mechanisms underlying this are different.*

We now have updated Table 1 to depict these aspects even more extensively:

Physiologic parameters	Interpretation
Spirometry	
FEF_{25-75} (corrected for FVC), L/s/L [13]	FEFs at 25-75% interval, or at 50% of expired lung

and FEF_{50} (corrected for FVC), L/s/L [13]	volumes are measurements of airflow obstruction in small-to-mid-caliber airways taken at low/mid expiratory lung volumes. When corrected for FVC, they are surrogate measures of the sizes of small-to-mid caliber airways relative to lung size, called dysanapsis. Dysanapsis is a characteristic favoring airways hyperresponsiveness.
Body plethysmography	
RV/TLC ratio, L/L [14]	Air trapping due to obstruction in both conducting small and peripheral airways
FRC, L [14]	Respiratory system resting volume as main determinant of whole airway static dimensions, and airway hysteresis
IOS	
R5-R20, kPa/L/s [15]	Respiratory Resistance of small-to-mid-sized conductive and peripheral airways
X5, kPa/L/s [15]	Respiratory System Reactance reflecting inertance and elasticity (capacitance), including small peripheral airways
AX, kPa/L [15]	Distensibility of the peripheral lungs (parenchyma + small peripheral airways)
MBNW	
Scond*VT, L^{-1} [16]	Index of convectional ventilation heterogeneity in peripheral conducting airways
Sacin*VT, L^{-1} [17]	Index of diffusive ventilation heterogeneity in most peripheral pre-acinar/acinar airways
Hyperresponsiveness	
Fall in FVC at PC_{20} or $PD_{20,}$ %[18,19]	Air trapping due to excessive bronchoconstriction or closure of small airways
CT scan parameters	
MLD ratio, I/E [25]	Ratio of mean lung density for inspiratory versus expiratory scans- a measure of air-trapping due to lung parenchyma inspiratory distension in the supine position
Lung volume ratio, cm^3 [22]	Ratio of CT-derived lung volume for inspiratory versus expiratory scans- a measure of air-trapping due to obstruction in both conducting small and peripheral airways in the supine position
VI-856, HU [21]	The voxel index < -856 Hounsfield Units from the expiratory scans, an index of expiratory air trapping

Legend to Table 1. Numbers in superscript refer to references used. IOS= impulse oscillometry; MBNW= Multiple breath nitrogen washout; CT= computed tomography, HU=Hounsfield Units

7. Table 1. What are at the units for FEF25-75 units when you correct for FVC? Is it the proportion of exhalation spent in the mid-exhalation? These should be interpretable enough for others to use.

Answer to comment 7: *The FEF25/75 values were corrected for level of FVC in statistical analyses, since this impacts on the FEF25-75 values. The unit of FEF25-75 is L/min. The FEF25/75 values were corrected for level of FVC in the statistical analyses, since this reflect the disproportionate growth between airway size and lung parenchyma that affect lung mechanics and airway reactivity. The FEF25-75 unit is L/s/L , see Table I, where the relevant unit was added to each measurement according to SI unit symbols.*

8. Table 4. I would include at the bottom of this table that the coefficients are per 1 unit increase in each parameter.

Answer to comment 8: *We have added this to the Table, as the Reviewer suggested.*

9. Table 4. FEF25-75 units are really important here too, since it is per unit change

Answer to comment 9: *We now have also added units for this variable*

10. Table 4. Do all of these results make sense to you? More exacerbations with higher R5-R20, higher RV/TLC ratio, lower FEF, lower Raw, lower height and females seems ok. What about the PC20 categories, I interpret the findings as very mild (and each category) has fewer exacerbations compared to normal. Are those results switched around? And in the second panel of results, are there really more consultations with a higher FEV1? Same comment for PC20 categories, they seem switched around here too.

Answer to comment 10: *When one measures hyperresponsiveness with PC20 or PD20 values, it means that a higher PD 20 reflects less severe hyperresponsiveness. Normal means that there was no hyperresponsiveness, so a very high value for PC20 or PD20.*

Individuals with a higher PC20 or PD20 have less frequent exacerbations, and conversely patients with lower PC20 or PD20, and thus more severe hyperresponsiveness have more frequent exacerbations.

To help interpretation we now have added the following to the Legend of the Table as follows:

"The coefficients are per 1 unit increase in each parameter. As example: the estimate of R5-R20 (kPa/L/s) for exacerbations is 2.894, one needs to calculate exp(2.894) = 18.065 and this means that for 1-unit increase of R5-R20 the mean number of exacerbations will increase by a factor of 18.07, holding other variables constant. Mild hyperresponsiveness means a higher PD_{20} or PC_{20} value. Patients with more severe hyperresponsiveness have more frequent exacerbations and unscheduled consultations. For abbreviations see Table 2

Specific comments from the editor:

1* The introduction is still slightly unclear as to what is being presented in this paper, and what else will be covered elsewhere in the future. There also seems to be some repetition with the methods section. I have suggested at the end of this email a possible revised introduction (that could be pasted in to the article). I have moved pieces of text from the current version and earlier rebuttal. This is just a suggestion to attempt to clarify the project, and would need to be checked for accuracy, so please amend as appropriate.

Answer to Editor's suggestion number 1*: We thank the Editor for this suggestion and changing to more alike we had proposed in an earlier version. We have adjusted the introduction as appropriate and have pasted this in the article as follows (changes underlined):*

"**Introduction**

Asthma is a prevalent obstructive airway disease that affects the entire bronchial tree. The small airways, defined by a diameter <2 mm and referred to as the "silent zone" of the lungs, contribute to the resistance in the airways of patients with obstructive airways disease[1]. This is of clinical importance since small airways can be inflamed in asthma and hence narrowed[2-4]. Small airway narrowing can also occur due to smooth muscle contraction after inhaling allergic and non-allergic irritants. Moreover, remodeling can affect small airway wall stiffness, thereby changing their distensibility[5].

Small airways dysfunction (SAD) has been postulated to exist at all severities of asthma, whereas some studies suggest that the prevalence increases with asthma severity [6,1]. However, it is still not clear what proportion of asthma patients suffers from SAD, and which tests or combination of tests best defines it. Lack of best practice is due to the fact that published studies investigating the small airways in asthma included only small-sized and/or relatively homogeneous populations regarding asthma severity, or only tested one or a few physiologic SAD measures [6-8].

The ATLANTIS (AssessmenT of smalL Airways involvemeNT In aSthma) study is a multinational 1-year prospective cohort study, including people with asthma of all severities and controls without airway disease. In this paper we present the baseline, cross-sectional data from ATLANTIS, ~~with the~~ aiming to identify which combination of biomarkers, physiologic testing and imaging approaches best measures the presence and extent of SAD in asthma, and their relationship with features of asthma. We assess SAD through a series of all available, clinically applicable, potential SAD tests, both for physiological and CT measures. The physiological tests may reflect abnormalities in different parts of the bronchial tree or different aspects of small airways dysfunction, providing different perspectives on SAD[9,10]. Lung imaging by CT scan can provide additional insight regarding SAD, but the relationship with physiologic measures of SAD in asthma has not been studied extensively and only in small groups; here we test both physiological and CT scan measures in a large cohort. In addition, we develop a score defining to what extent SAD is present in each individual patient and assess its usefulness for prediction of asthma severity, control, quality of life and history of exacerbations. In future papers (not presented here), we will report the longitudinal data from ATLANTIS and aim to validate the SAD score over time, we will develop and validate a Small Airways Dysfunction Tool (SADT), a questionnaire as an easy measure to suggest SAD, and we will assess which direct and indirect measures of inflammation best discriminate between the large and small airways' compartments, with bronchial and transbronchial biopsies, in a smaller subset of participants."

2* At the last round of review, one of the reviewers requested that the strengths and

limitations weren't at the beginning of the section, and we would prefer this information comes later in the discussion. Although we use the STROBE checklist to ensure that all components are included, in our guidance for authors (http://www.thelancet.com/pb/assets/raw/Lancet/authors/tlrm-info-for-authors.pdf) we indicate "The Discussion section should contain a full description and discussion of the context." We would suggest starting with a summary of the main findings as you have done, followed by a section relating results to earlier work (moving text from later in the discussion).

Answer to Editor's suggestion number 2: *We have followed the Editor's suggestion and rearranged the Discussion as was suggested.*

3* A minor point, but in the first round of review, reviewer 3 raised the issue of racial differences. Although there are insufficient data for analyses by race, could the main manuscript note (in the results section describing patient characteristics), that the large majority of people included were white (88% and 96% in asthmatic and the control groups). Although this is mentioned in the supplement, it could be of interest to readers of just the main paper.

Answer to Editor's suggestion number 3: *We have added the percentages as the Editor has suggested as follows (changes underlined):*

"Participants

Baseline characteristics are shown in Table 2, Table 3 (asthma only) and Supplemental Table 2. Gender, age and smoking habits were comparable between asthma and control participants; the large majority of people included were of Caucasian descent (88% and 96% in asthma and control participants respectively).

General editorial comments:

* Our research articles (that are not RCTs) are usually up to 3500 words with up to 30 references, and your article is currently 4462 words with 49 references. Although we can be slightly flexible, and we appreciate that additions have been made to the article during the peer-review process as requested by reviewers, please consider these limits when making your final revisions before acceptance. If there is text that could be shortened, or references that could be removed, please change at this stage. I have suggested some cuts to the introduction below, and the discussion could also be an area to trim.

Answer to Editor's General comment 1: *We have reduced the length of the manuscript an tried to reduce also the number of References. However, given the questions and suggestions by the reviewers, and particularly Reviewer 4 we had to add quite a few references and especially to Table1. We have rewritten the Discussion, and now have reduced the number of words from 4463 to 4364 in total, and the number of references from 49 to 44.*

* The study title should include a descriptor—ie, randomised trial, case-control study, prospective analysis, population-based study etc… In this case the ATLANTIS study is a prospective cohort study, but in this paper baseline data are being reported. Could we suggest a revision to the title something along the lines of: "Exploring the relevance and extent of small airways dysfunction in asthma: baseline data from the AssessmenT of smalL Airways involvemeNT In aSthma (ATLANTIS) prospective cohort study"

Answer to Editor's General comment 2: *We have rephrased the Title of the manuscript as follows to capture the prospective cohort study as follows:*

"Exploring the relevance and extent of small airways dysfunction in asthma: baseline data from the AssessmenT of smalL Airways involvemeNT In aSthma (ATLANTIS) prospective cohort study"

3* Please check with your co-authors, and confirm, that all names are spelt correctly, and affiliations listed correctly. We will not correct names and affiliations after publication of your article.

Answer to Editor's General comment 3: *All author's names have been spelt correctly*

4* Please supply (after author names on the title page) one preferred degree per author and indicate in the authorship if any authors are full professors.

Answer to Editor's General comment 4: *We now have below added one preferred degree per author and have added full professorships.*

Postma DS[1], MD Prof, Brightling C[2], MD Prof, Baldi S[2,11], MD PhD, Van den Berge M[1] , MD, Fabbri LM[3,4], MD Prof, Gagnatelli A[5], PhD, Papi A[6], MD Prof, Van der Molen T[7], MD, Prof, Rabe KF[8], MD Prof, Siddiqui S[2],MD, Singh D[9], MD Prof, Nicolini G[5], PhD, Kraft M[10] , MD Prof, and the ATLANTIS study group*

5* Please give full first names for all authors:

Answer to Editor's General comment 5: *The first names of authors are as follows:*

Postma Dirkje S[1], Brightling Chris[2], Baldi Simonetta[2,11], Van den Berge Maarten[1], Fabbri Leo M[3,4], Gagnatelli Alessandra[5], Papi Alberto[6], Van der Molen Thys[7], Rabe Klaus F[8], Siddiqui Salman[2], Singh Dave [9], Nicolini Gabriele[5], Kraft Monica[10] and the ATLANTIS study group*

6 * Summary: We have requested additions to the abstract below. At this stage, please do not worry about the word length of the abstract - accuracy and completeness here are key:

-Background: Please include the aim of this study in this section.
-Methods: Please add a brief summary of the main patient characteristics (ie, main entry criteria)

-Interpretation: We suggest replacing 'Further work is needed' with information about the follow-up of the ATLANTIS study.

Answer to Editor's General comment 6: *Here we have followed your suggestions and those of Reviewer #4 and changed the abstract as follows (changes underlined). This consequently has lengthened the abstract considerably:*

Summary

Background

Small airways dysfunction (SAD) is well-recognized in asthma, yet its role in asthma severity and asthma control is unclear. Our study aimed to assess which (combination of) biomarkers, physiological testing and imaging markers best measures the presence and extent of SAD in asthma.

Methods

This multinational observational study investigated participants without and with asthma (GINA severity stage 1-5). Asthma inclusion criteria were: 1) age 18-65 years; 2) clinical asthma diagnosis > 6 months, confirmed by a chest physician 2, supported by objective evidence of any of the following at the baseline visit or in the previous 5 years: a) positive airway hyperresponsiveness to methacholine, ***or*** b) positive reversibility ($\Delta FEV_1 \geq$ 12% and ≥ 200 mL within 30 minutes after 400 µg of salbutamol pMDI with or without a spacer ***or c)*** PEF variability >20%, measured during 7 days ***or*** d) documented reversibility after a cycle (e.g. 4 weeks) of maintenance anti-asthma treatment; 3) stable asthma on any previous regular asthma treatment ("rescue" β2-agonists alone included) at a stable dose for > 8 weeks before baseline; 4) lifetime smoking ≤ 10 pack-years. They underwent spirometry, body plethysmography, impulse oscillometry (IOS), Multiple Breath Nitrogen Washout (MBNW), computed tomography (CT) and questionnaires. Structural equation modeling (SEM) was applied in asthma to assess the contribution of all physiological and CT parameters to SAD. With SEM, we defined a clinical-SAD and CT-SAD score. Asthma subjects were classified in SAD groups using model-based clustering. Asthma severity, control and health care utilization in the past year were compared with the SAD scores and SAD groups.

Findings

We investigated 773 asthma and 99 control participants (median [interquartiles] age 46 [34, 54] and 41 [29, 52] years, 58% and 57% females, respectively). All physiologic measures contributed to the clinical SAD model with SEM analysis. The prevalence of SAD in asthma was dependent on the measure used and lowest with MBNW Sacin that reflects ventilation heterogeneity in the most peripheral, pre-acinar/acinar airways. IOS and spirometry, reflecting dysfunction of small-to-mid-sized airways, contributed most to the Clinical-SAD score and differentiated the two SAD Groups. Clinical-SAD Group1 (n=452) had "milder" SAD, i.e. comparable ventilation heterogeneity in pre-acinar/acinar airways values (MBNW Sacin) with controls. Group2 (n=312) had more abnormal physiologic SAD measures than Group1, particularly IOS and spirometry, and more severe asthma (asthma control, treatments, exacerbations, quality of life). Clinical-SAD scores were higher in Group2 ("more severe" SAD) and related to asthma control, severity, and exacerbations. Clinical-SAD and CT-SAD scores did not significantly correlate.

Interpretation

SAD is a complex and silent signature of asthma, which is likely to be directly or indirectly captured by combinations of physiologic tests: spirometry, body plethysmography, IOS, and MBNW. SAD is present across all asthma severity and particularly in severe disease. The clinical classification of SAD in two groups, i.e. a "milder" and "more severe" SAD group, by the easy-to-conduct measures IOS and spirometry, is meaningful given its association with GINA asthma severity stages, asthma control, quality of life, and exacerbations. The longitudinal part of ATLANTIS will show the relevance of the SAD score for future risks in asthma, and additionally which parameter best associates with future asthma control. Moreover, we will report on development of a Small Airways Dysfunction Tool (SADT), a questionnaire as an easy measure to suggest SAD, and on the measures of inflammation that best discriminate between the large and small airways' compartments, with bronchial and transbronchial biopsies, in a smaller subset of participants.

7 * You've noted in your rebuttal that your article confirms to the STROBE guidance, and we ask authors to complete this checklist and submit it with their revised article. Please complete the checklist, available here:
STROBE - Observational studies —
http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(07)61602-X/fulltext
For more info: http://www.equator-network.org/

Answer to Editor's General comment 7: *We have inserted the Strobe table below and have added all pages. NA means Not Applicable*

Table The STROBE statement—checklist of items that should be addressed in reports of observational studies

	Item	Recommendation	Reported on manuscript page
Title and abstract			
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Page1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 3
Introduction			
Background/rationale	2	Explain the scientific background	Pages 7-8

	Item	Recommendation	Reported on manuscript page
		and rationale for the investigation being reported	
Objectives	3	State specific objectives, including any prespecified hypotheses	Pages 7-8
Methods			
Study design	4	Present key elements of study design early in the paper	Pages 7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 9
Participants	6	(a) *Cohort study*—give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 9
		Case-control study—give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	NA
		Cross-sectional study—give the eligibility criteria, and the sources and methods of selection of participants	Page 9
		(b) *Cohort study*—for matched	NA

	Item	Recommendation	Reported on manuscript page
		studies, give matching criteria and number of exposed and unexposed	
		Case-control study—for matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Pages 9-11 and supplement
Data sources/measurement	8 *	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Pages 9,10 and supplement
Bias	9	Describe any efforts to address potential sources of bias	Page 9
Study size	10	Explain how the study size was arrived at	Supplement
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	Page 11 and Supplement
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Page 11 and Supplement

	Item	Recommendation	Reported on manuscript page
		(b) Describe any methods used to examine subgroups and interactions	Page 11 and Supplement
		(c) Explain how missing data were addressed	Reference 25, and Page 11, and Page 9 of Supplement
		(d) *Cohort study*—if applicable, explain how loss to follow-up was addressed	NA
		Case-control study—if applicable, explain how matching of cases and controls was addressed	NA
		Cross-sectional study—if applicable, describe analytical methods taking account of sampling strategy	All data were used at baseline, Supplement
		(e) Describe any sensitivity analyses	NA
Results			
Participants	13 *	(a) Report the numbers of individuals at each stage of the study—eg, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1

	Item	Recommendation	Reported on manuscript page
		(b) Give reasons for non-participation at each stage	Figure 1
		(c) Consider use of a flow diagram	
Descriptive data	14 *	(a) Give characteristics of study participants (eg, demographic, clinical, social) and information on exposures and potential confounders	Page 12, Tables 2 and 3, and Supplemental Table 2
		(b) Indicate the number of participants with missing data for each variable of interest	Figures 2 and 5, and Supplement
		(c) *Cohort study*—summarise follow-up time (eg, average and total amount)	NA
Outcome data	15 *	*Cohort study*—report numbers of outcome events or summary measures over time	NA
		Case-control study—report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—report numbers of outcome events or summary measures	Figures 2 and 5 and Supplement
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg,	Page 11 and Supplement

	Item	Recommendation	Reported on manuscript page
		95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg, analyses of subgroups and interactions, and sensitivity analyses	Page 11, Page 15
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 17
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Pages 18 and 19
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 22

	Item	Recommendation	Reported on manuscript page
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 12

*** Give such information separately for cases and controls in case-control studies, and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies. An explanation and elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the websites of *PLoS Medicine, Annals of Internal Medicine,* and *Epidemiology*). Separate versions of the checklist for cohort, case-control, and cross-sectional studies are available on the STROBE website.**

8 * Please report means with SDs (e.g. mean SAD score, mean age in discussion).

Answer to Editor's General comment 8: *We now have adjusted the Discussion and report means accompanied by SD values and medians with interquartile ranges.*

9* Lancet style is to provide p values to two significant figures, unless p<0.0001 (if this is the case, then please revise to the latter). You need only give the p-value to 2 decimal places for non-significant results (ie p=0.87).

Answer to Editor's General comment 9: *We have followed the suggestions of the Editor. However, when reading Lancet Respiratory Medicine articles, we noted that many of them show figures with three significant numbers, being 0.057 for instance . We wondered whether the p<0.0001 instead was p< 0.001? It is important to know if significances are extremely significant or borderline. And we agree that hence it is sufficient to have two significant numbers. Thus, we changed accordingly 0.057 into 0.06, and 0.029 in 0.03. However, this is not the case for values like 0.001 and 0.002. We now have adjusted throughout the manuscript all Tables and changed all other numbers to two significant values if < 0.001.*

We have also changed this in the Supplemental Tables.

We hope that this is what Lancet Respiratory Medicine wishes and so the readers can interpret our data.

10 * As I am sure you are aware, the Lancet group are very supportive of protocol-based research and so encourage authors to post the protocol document on a publicly accessible website; a margin link to the website will then be put in the paper. Would you like to do this for your protocol? Perhaps the link to the ERJ paper? If so, please send us the protocol link with your final corrections. Please note that if you do wish to do this then the weblink should not be temporary.

Answer to Editor's General comment 10:

We hereby provide a link to the paper in the ERJ. https://www.ncbi.nlm.nih.gov/pubmed/26028618, and we had already provided a Link to the full study, as follows

https://clinicaltrials.gov/ct2/show/NCT02123667?term=NCT02123667&rank=1

We hope this is what Lancet Respiratory Medicine is needing

* Lancet style is to have a 'Role of the funding source' at the end of the methods. The following points need to be addressed in the "Role of the funding source" statement:

* The role of the sponsors in the study design.

* The role of the sponsors in the collection, analysis, or interpretation of the data.

* The role of the sponsors in the writing of the report.

* Those who had access to the raw data (by author initials). If the funding source had no role then this should be stated. Please also add to this section (if true): "The corresponding author had full access to all of the data and the final responsibility to submit for publication."

Answer to Editor's General comment 10:

We now have added the following after the Methods section:

Role of the Funding source
Chiesi Farmaceutici SpA financed the study, contributed to the set-up of the study which was designed by DP, MK, CB, MvdB, LF, AP, TvdM, KR, SS and DS. Chiesi Farmaceutici SpA contributed to interpretation of the study and approved the submitted manuscript. Data collection and management was done by Cromsource and data were analysed by CROS NT. All co-authors discussed and interpreted the data. The first draft of the report was written by DP, CB and MK; DP collated input from all co-authors. DP and MK had access to raw data. The corresponding author had full access to all of the data and the final responsibility to submit the initial and revised manuscript.

11* At the beginning of the methods section, please give the exact dates here (if known)—ie, day, month, year.
Answer to Editor's General comment 11: We now have added this as follows:

“Participants were recruited (first patient in June 30, 2014 and last patient out March 3, 2017) from general practitioners, chest physician’s databases and by advertisements in 29 centers across 9 countries worldwide.”

12* In the Research in context section, 'Evidence before this study', please include a description of all the evidence that the authors considered before undertaking this study. Authors should state: the sources (databases, journal or book reference lists, etc) searched; the criteria used to include or exclude studies (including the exact start and end dates of the search), which should not be limited to English language publications; the search terms used; the quality (risk of bias) of that evidence; and the pooled estimate derived from meta-analysis of the evidence, if appropriate.

Answer to Editor’s General comment 12: *We now have added the following to the Research in Context section*:

“We searched PubMed for studies in asthma, including the terms asthma, adult, and small airways, and published between database inception and April 2018, using spirometry and any combination of body plethysmography, impulse oscillometry (IOS; including R5-R20 values) and Multiple Breath Nitrogen Washout (MBNW) measures, and similar terms in addition to CT scans.”

13 * ICMJE Conflicts of Interest form: We have received all forms except from Dr Siddiqui.
Answer to Editor’s General comment 13: *We now have added the form with the name on it.*

14* Please ensure there is a conflict of interest statement to the end of your paper, as per Lancet style. These statements should exactly match those given on your signed conflict of interest forms. If there are none then please state "The authors declared no conflicts of interest" or "The other authors declared no conflicts of interest."
Answer to Editor’s General comment 14: *We have now added the following text after the Discussion section as follows:*

Declaration of interest

D.P. reports that the University of Groningen has received money regarding a research grant from Astra Zeneca, Chiesi, Genentec, GSK and Roche, regarding consultancies from Astra Zeneca, Chiesi, and GSK, outside the submitted work. CB reports grants and personal fees from Chiesi, grants from AirPROM, during the conduct of the study; grants and personal fees from GlaxoSmithKline, AstraZeneca/Medimmune, Boehringer Ingelheim, Novartis, Chiesi, Roche/Genentech, personal fees from Vectura, Theravance, PreP, Gilead, Sanofi/Regeneron Teva, grants from Pfizer and Mologic, personal fees from Gossamer and 4DPharma, outside the submitted work. SB reports personal fees from Chiesi SAS FRANCE, during the conduct of the study; personal fees from employment, outside the submitted work. MvdB reports research grants paid to University from Chiesi, Teva Pharma, GlaxoSmithKline, outside the submitted work. LF reports personal fees, non-financial support and other from Chiesi Farmaceutici, during the conduct of the study; grants, personal fees and non-financial support from Boehringer Ingelheim, Chiesi Farmaceutici, GlaxoSmithKline, Merck Sharp & Dohme, Takeda, AstraZeneca, Novartis,Menarini; personal fees and non-financial support from Pearl Therapeutics and Mundipharma ,personal fees from Zambon, outside the submitted work.

AG and GN report employment by Chiesi Farmaceutici S.p.A. which sponsored the study. AP reports grants and personal fees from Chiesi Pharmaceuticals, during the conduct of the study; grants, personal fees, non-financial support and other from Chiesi, Astrazeneca, GlaxoSmithKline, Boehringer Ingelheim, Mundipharma and personal fees and non-financial support from Menarini, Pfizer, Novartis, Zambon, outside the submitted work. TvdM reports grants and personal fees from Astra Zeneca, TEVA, GSK, personal fees from Boehringer Ingelheim, outside the submitted work; and From 1 June 2017 T van der Molen is a part-time employee of GSK. KR reports personal fees from AstraZeneca, Boehringer Ingelheim, Novartis, Sanofi, Teva, Intermune, Chiesi Pharmaceuticals, Berlin Chemie and grants from Ministry of Education and Science, Germany outside the submitted work. SS reports grants from Chiesi onulus foundation, Sir Jule Thorne Trust, Medical Research Council/EPSRC NAPP, NIHR UK, and personal fees from AZ, GSK, Boehringer Ingelheim, Novartis, Mundipharma, Owlstone, outside the submitted work. D Singh reports grants and personal fees from Chiesi, AstraZeneca, Boehringer Ingleheim, GlaxoSmithKline, Glenmark, Merck, Menarini, Mundipharma, Novartis, Peptinnovate, Pfizer , Pulmatrix, and personal fees from Cipla, Apellis, Genentech, Skyepharma, Teva, Therevance, and Verona, outside the submitted work. MK reports grants from NIH, Chiesi, Sanofi, personal fees from Elsevier, during the conduct of the study.

15 * Authorship forms: We have received all forms except from Dr Siddiqui.
Answer to Editor's General comment 15: *We now have added the form with the appropriate name.*

16 * Please ensure there is an Author contributions section to the end of your paper before the references, as per Lancet style. These statements should exactly match those given on your signed forms. Authors should be referred to by their initials in this section

Answer to Editor's General comment 16: *We now have added the following:*

"Contributors

DP, MK, CB, MvdB, LF, AP, TvdM, KR, SS and DS designed the study. DP, MK, CB, MvdB, LF, GA, AP, TvdM, KR, SS, NG and DS discussed and interpreted the data. The first draft of the paper was written by DP, CB and MK; DP collated input from all co-authors who reviewed all versions of the manuscript. DP and MK had access to raw data. The corresponding author had full access to all of the data and the final responsibility to submit the initial and revised manuscript."

17 * All web references should have the date they were last accessed (e.g. ref 11).
Answer to Editor's General comment 17: *We have added the date of last access to references with web sites.*

18* Please supply figures in an editable format, such as high-resolution EPS format. Where applicable please export directly from your statistical package if possible.

Answer to Editor's General comment 18: *we have submitted all figures as appropriate.*

19 * It is not Lancet policy to edit or style supplementary material for the web; however, this

material will still be hosted on our website as a pdf of the author supplied file. Please style your supplementary material as per the guidelines found at http://www.thelancet.com/pb/assets/raw/Lancet/authors/tlrm-info-for-authors.pdf. Please note that we will be unable to correct any errors in the webappendix following publication; as such, please check carefully when submitting. Please supply the webappendix as a single PDF file, with the pages paginated.

Answer to Editor's General comment 19: *We have provided an accurate Supplemental file with pages numbered.*

20 * It is not TLRM policy to include investigator lists in the main paper; instead, these will be published in an online appendix that is linked to the paper. Please move the list to the appendix.

Answer to Editor's General comment 20: *We have deleted the list of investigators from the main paper as requested and added it in the Supplement.*

21 * If you would like the contributors from the ATLANTIS study group to be included in PubMed, we're now required to supply a separate list of the group members in a specific format if we want these names to be shown on PubMed. (This is in addition to the list of names and affiliations required by the journal to be listed at the end of the paper or in the appendix.) If relevant, to ensure that the information we supply to PubMed is accurate and complete, please upload with your revision a list of all study group members whose names should appear on PubMed, presented as a two-column table in Word. First and middle names or initials should be placed in the first column, and surnames in the second column. Names should be ordered as you wish them to appear on PubMed. The table will not be included in the paper itself - it's simply used to make sure that PubMed adds the names correctly.

Answer to Editor's General comment 21: *The list of collaborators for PubMed is as follows:*

name	surname
Emilio	Pizzichini
Alberto	Cukier
Rafael	Stelmach
Ronald	Olivenstein
Qingling	Zhang
Philipp	Badorrek
Christian	Gessner
Nicola	Scichilone
Alfredo	Chetta
Pierluigi	Paggiaro
Stefano	Milleri
Mariella	D'Amato
Antonio	Spanevello
Maria Pia	Foschino
Willem Germen	Boersma

Marielle	Broeders
J.Sebastiaan	Vroegop
Vicente	Plaza Moral
Ratko	Djukanovic
Omar	Usmani
Robert	Schilz
Richard	Martin
Nicola	Hanania

Answer to Suggested edits to the introduction:

We have changed the introduction as little as possible and only changed where clarity was needed. The changes from the text provided have been underlined.

"Asthma is a prevalent obstructive airway disease that affects the entire bronchial tree. The small airways, defined by a diameter <2 mm and referred to as the "silent zone" of the lungs, contribute to the resistance in the airways of patients with obstructive airways disease[1]. This is of clinical importance since small airways can be inflamed in asthma and hence narrowed[2-4]. Small airway narrowing can also occur due to smooth muscle contraction after inhaling allergic and non-allergic irritants. Moreover, remodeling can affect small airway wall stiffness, thereby changing their distensibility[5].

Small airways dysfunction (SAD) has been postulated to exist at all severities of asthma, whereas some studies suggest that the prevalence increases with asthma severity [6,1]. However, it is still not clear what proportion of asthma patients suffers from SAD, and which tests or combination of tests best defines it. Lack of best practice is due to the fact that published studies investigating the small airways in asthma included only small-sized and/or relatively homogeneous populations regarding asthma severity, or only tested one or a few physiologic SAD measures [6-8].

The ATLANTIS (AssessmenT of smalL Airways involvemeNT In aSthma) study is a multinational 1-year prospective cohort study, including people with asthma of all severities and ~~healthy~~ controls without airway disease. In this paper we present the baseline, cross-sectional data from ATLANTIS, aiming to identify which combination of biomarkers, physiologic testing and imaging approaches best measures the presence and extent of SAD in asthma, and their relationship with features of asthma. We assess SAD through a series of all available, clinically applicable, potential SAD tests, both for physiological and CT measures. The physiological tests may reflect abnormalities in different parts of the bronchial tree or different aspects of small airways dysfunction, providing different perspectives on SAD[9,10]. Lung imaging by CT scan can provide additional insight regarding SAD, but the relationship with physiologic measures of SAD in asthma has not been studied extensively and only in small groups; here we test both physiological and CT scan measures in a large cohort.
In addition, we develop a score defining to what extent SAD is present in each individual patient and assess its usefulness for prediction of asthma severity, control, quality of life and history of exacerbations. In future papers (not presented here), we will report the longitudinal data from ATLANTIS and aim to validate the SAD score over time, we will develop and

validate a Small Airways Dysfunction Tool (SADT), a questionnaire as an easy measure to suggest SAD, and we will assess which direct and indirect measures of inflammation best discriminate between the large and small airways' compartments, with bronchial and transbronchial biopsies, in a smaller subset of participants[9].“

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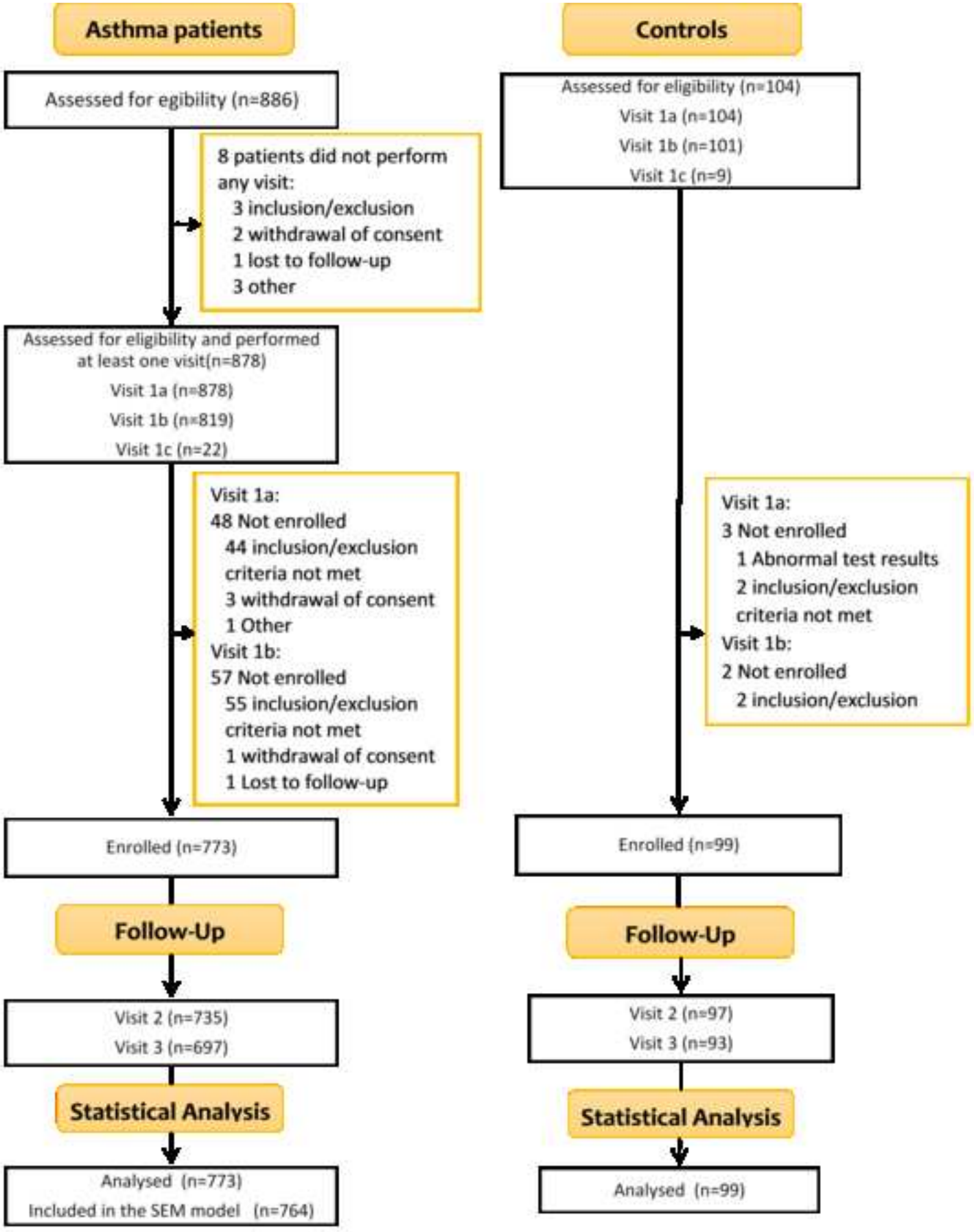

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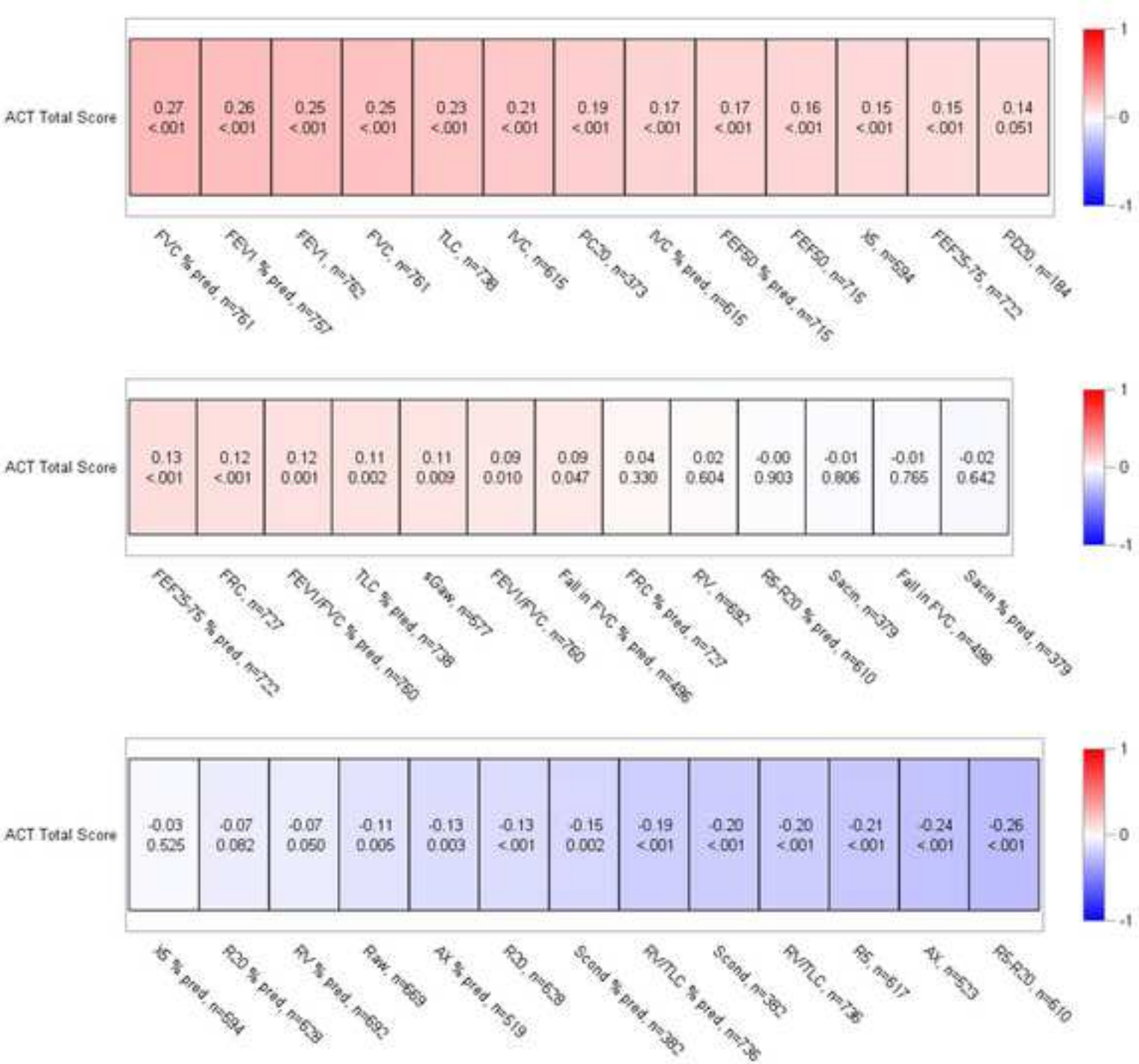

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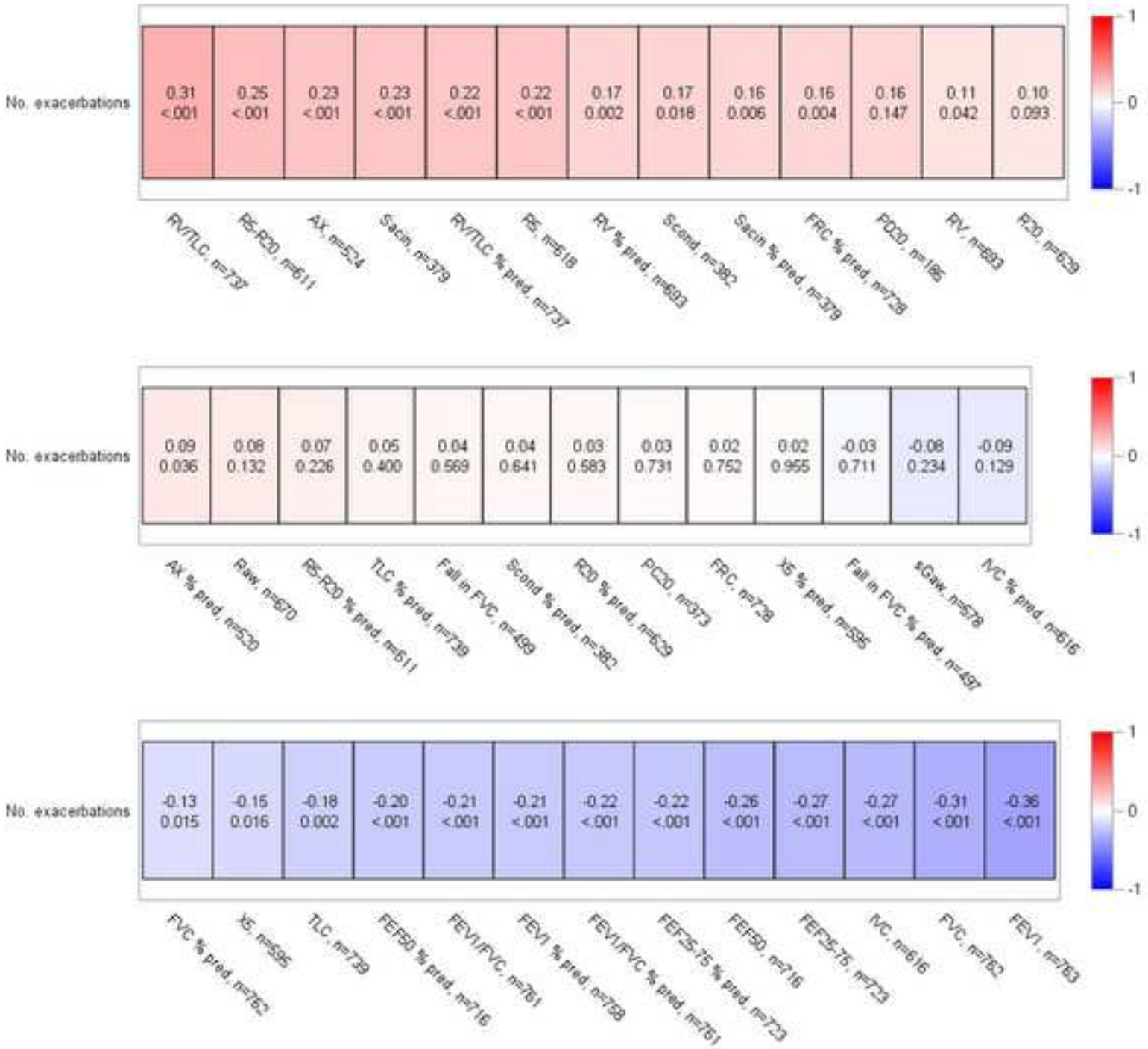

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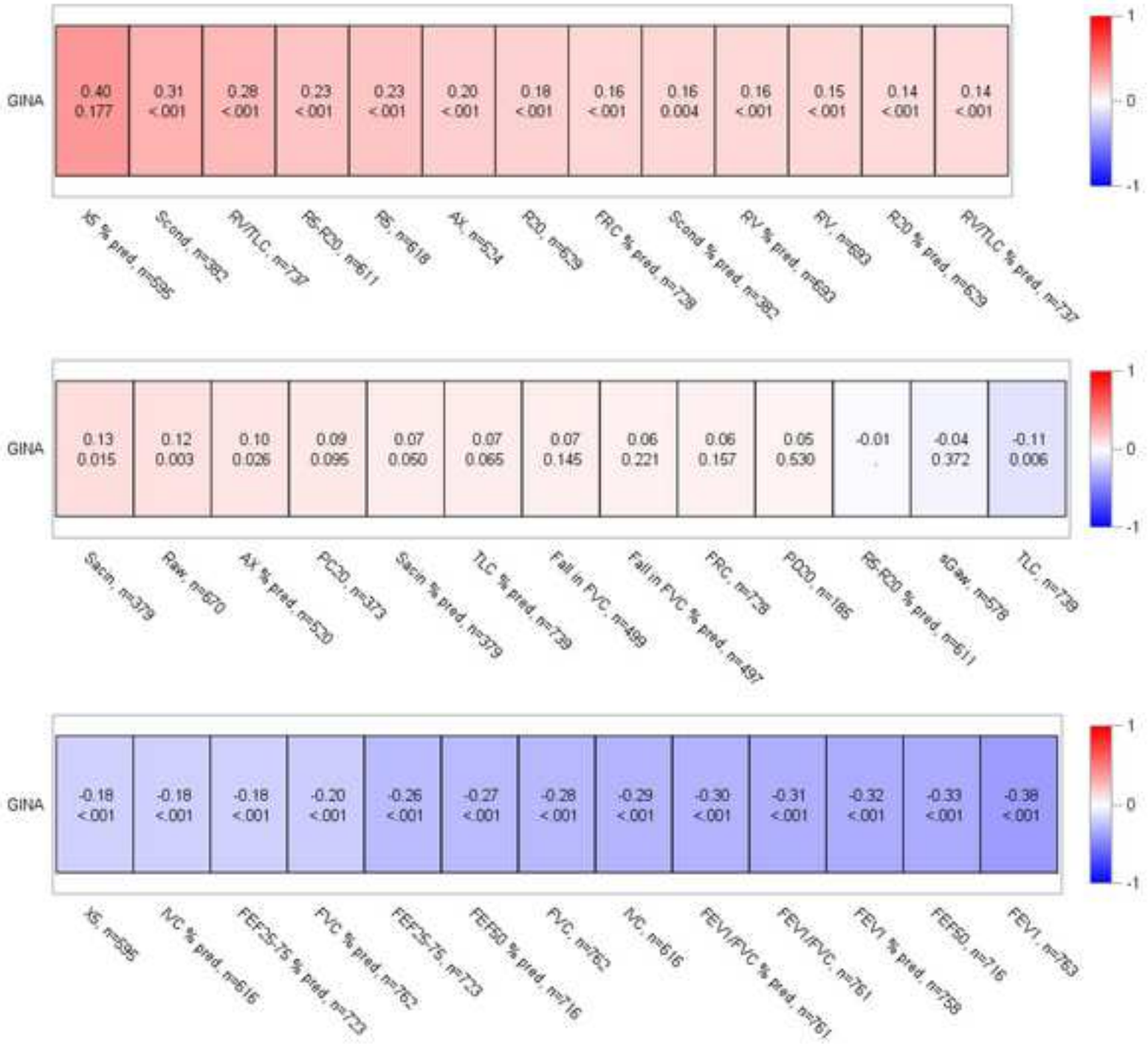

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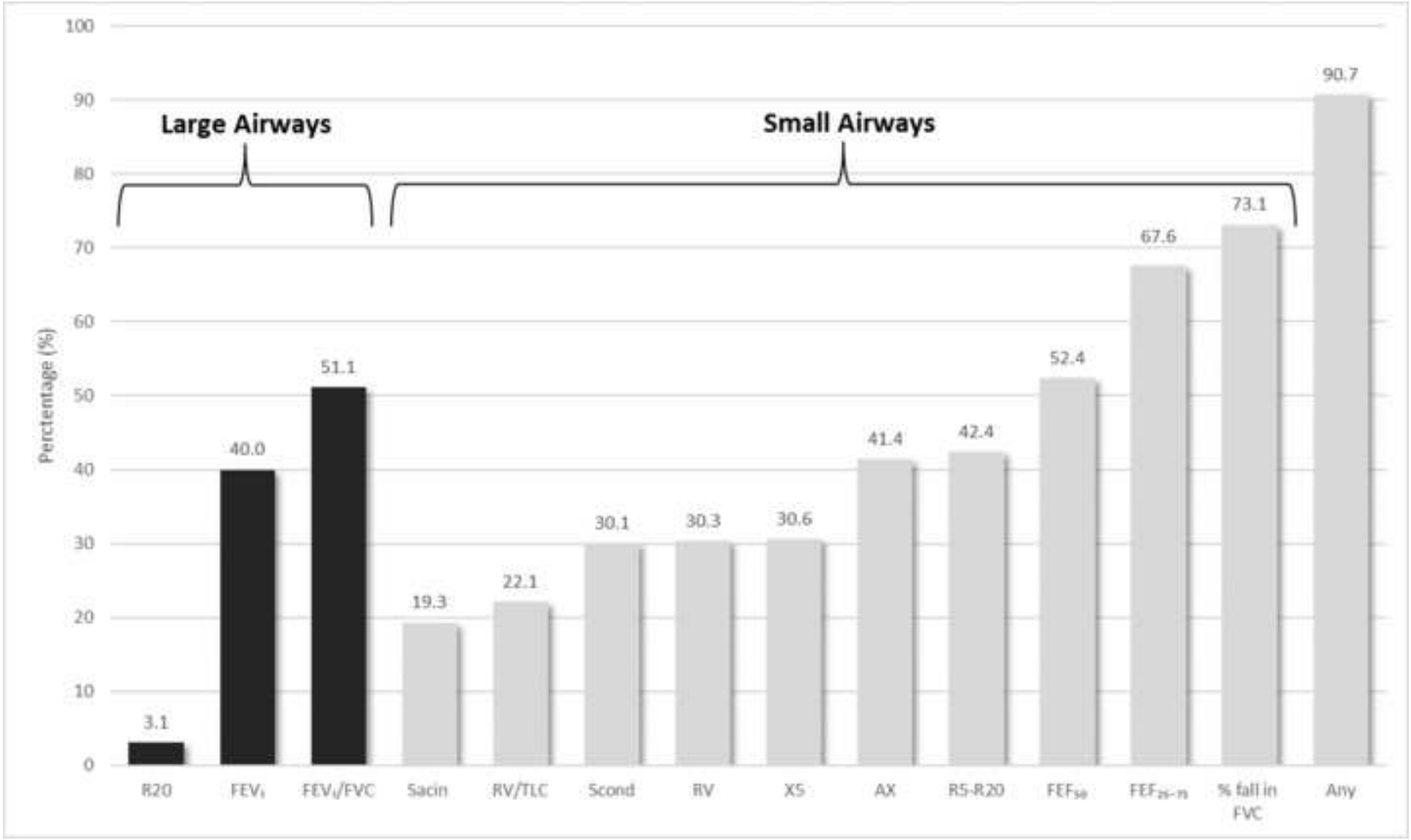

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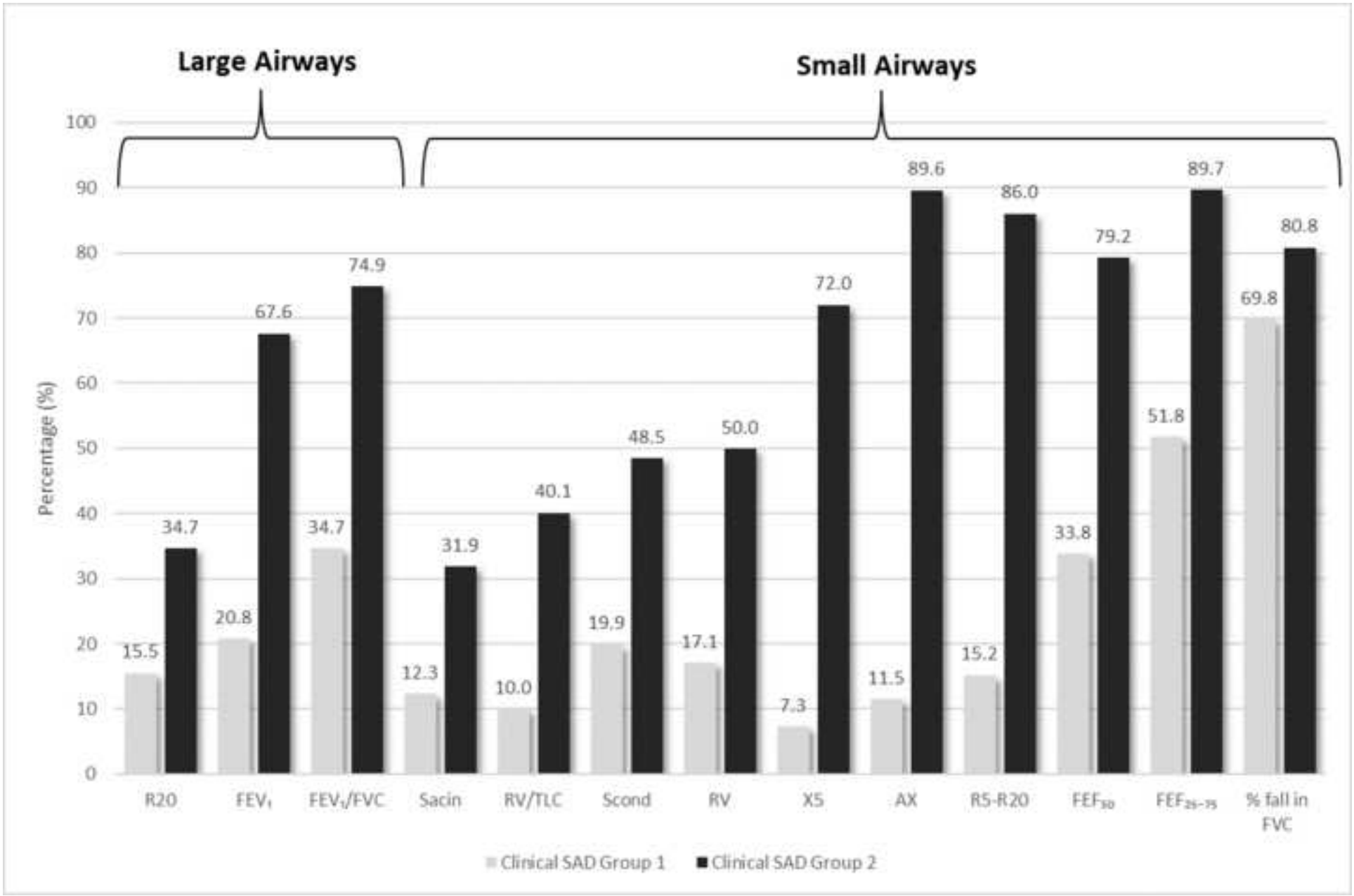

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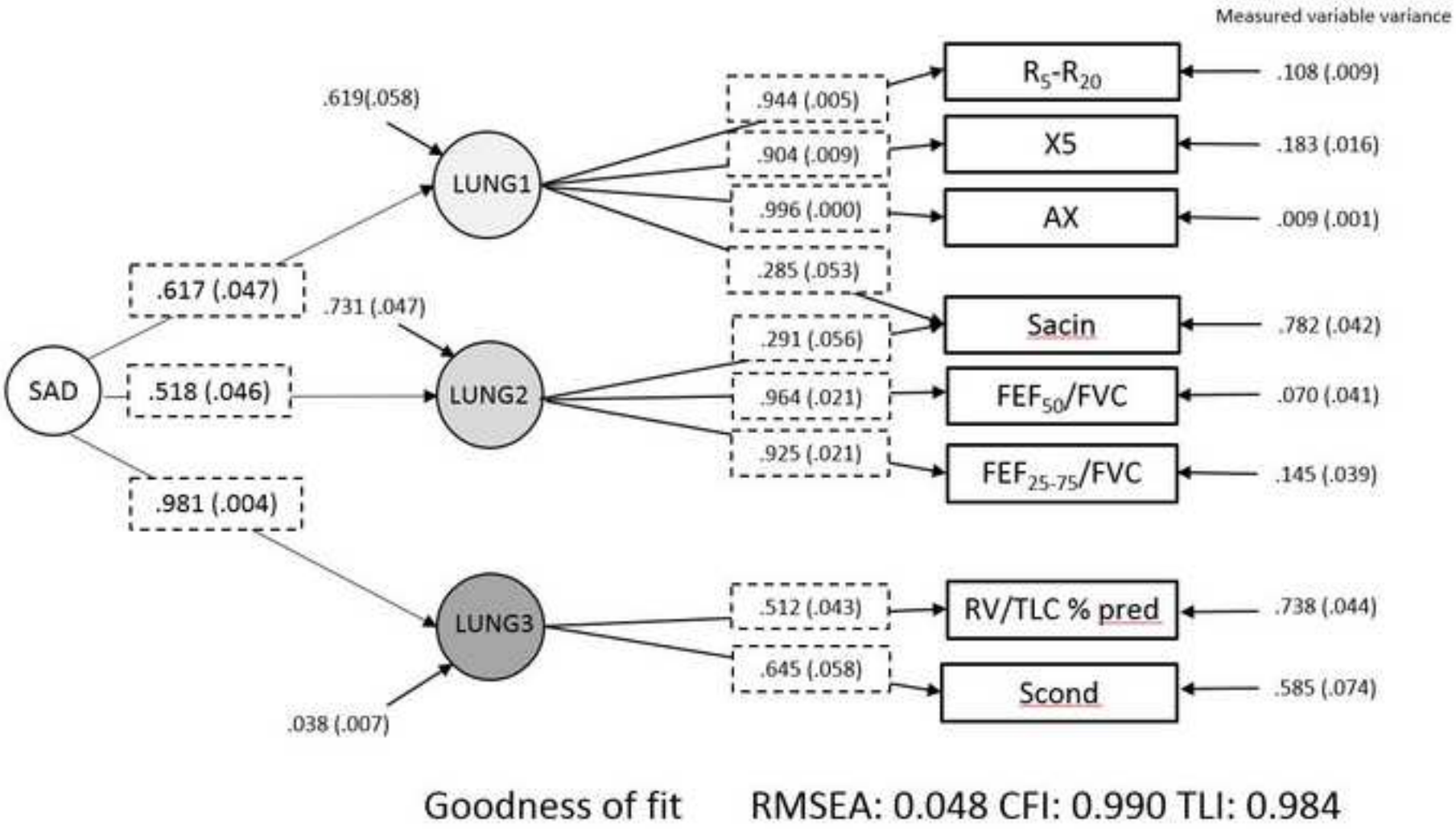

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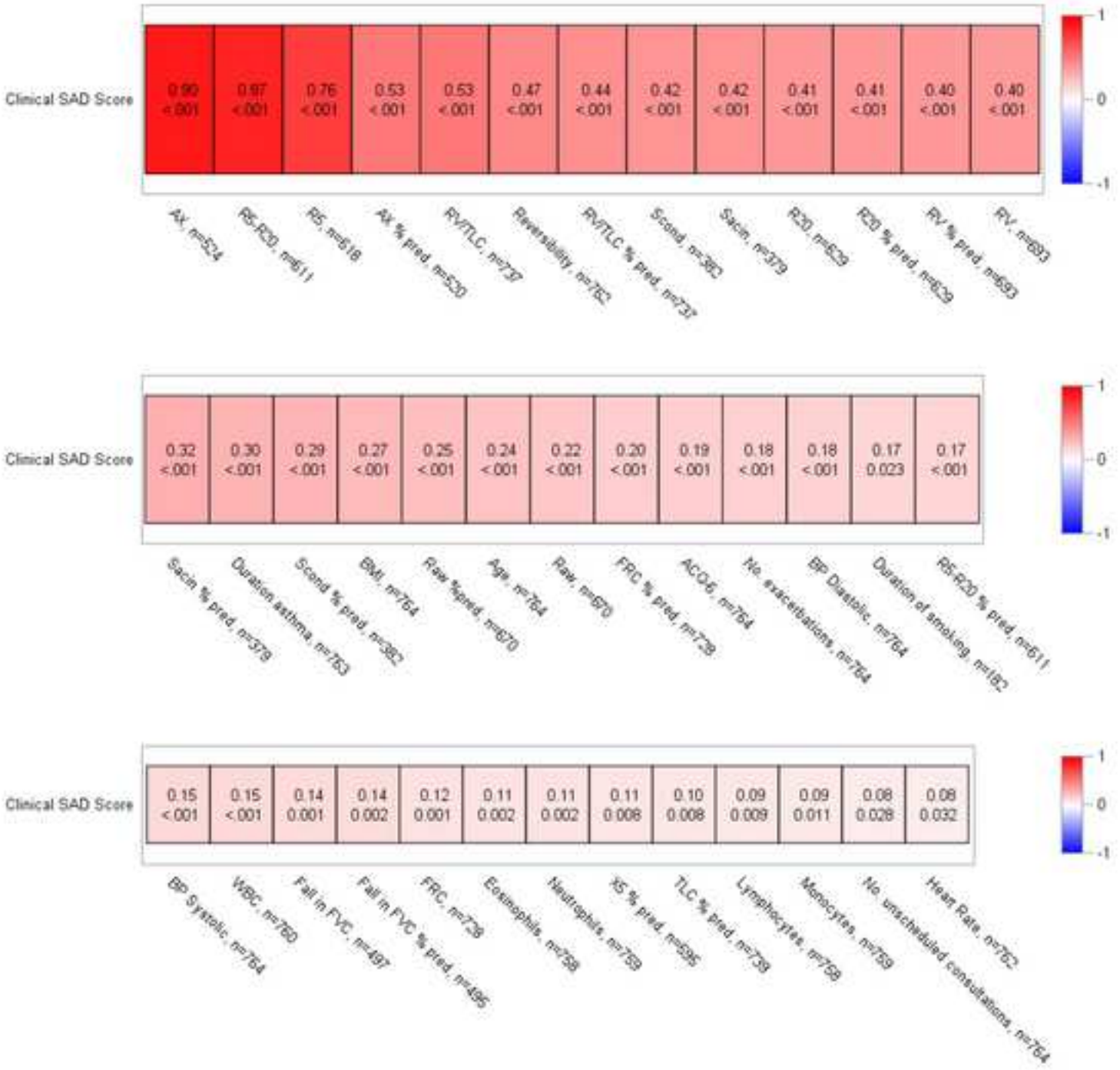

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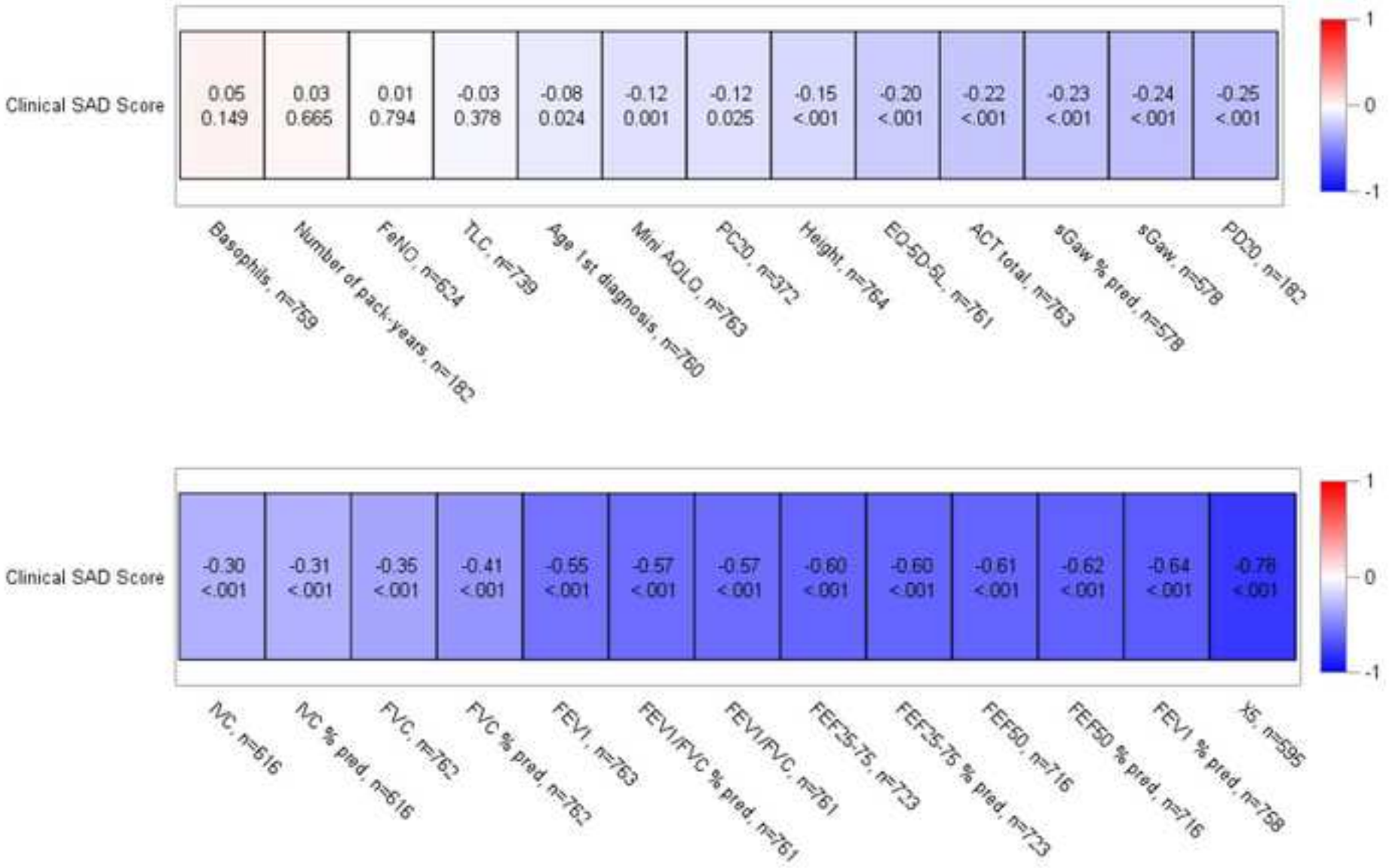

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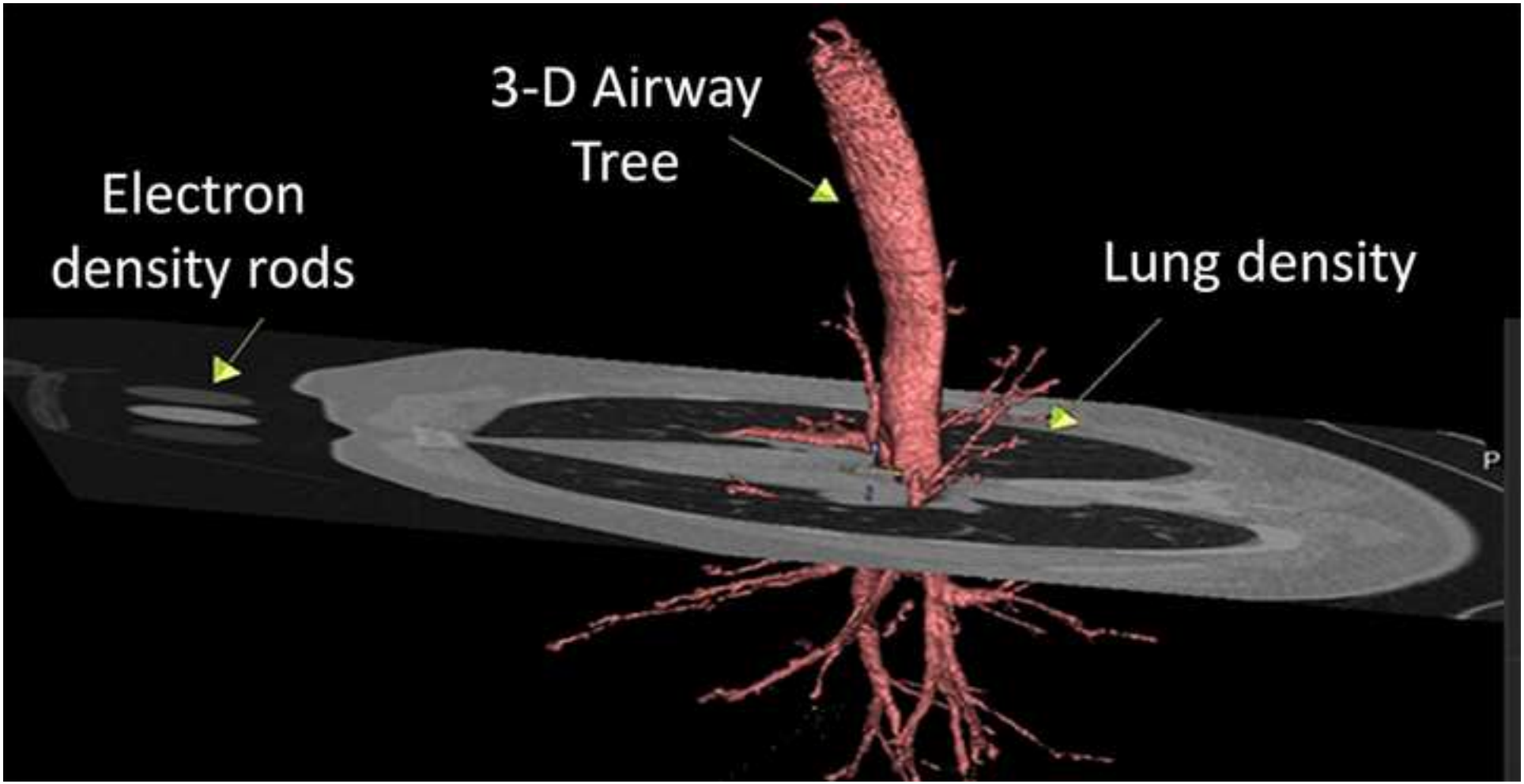

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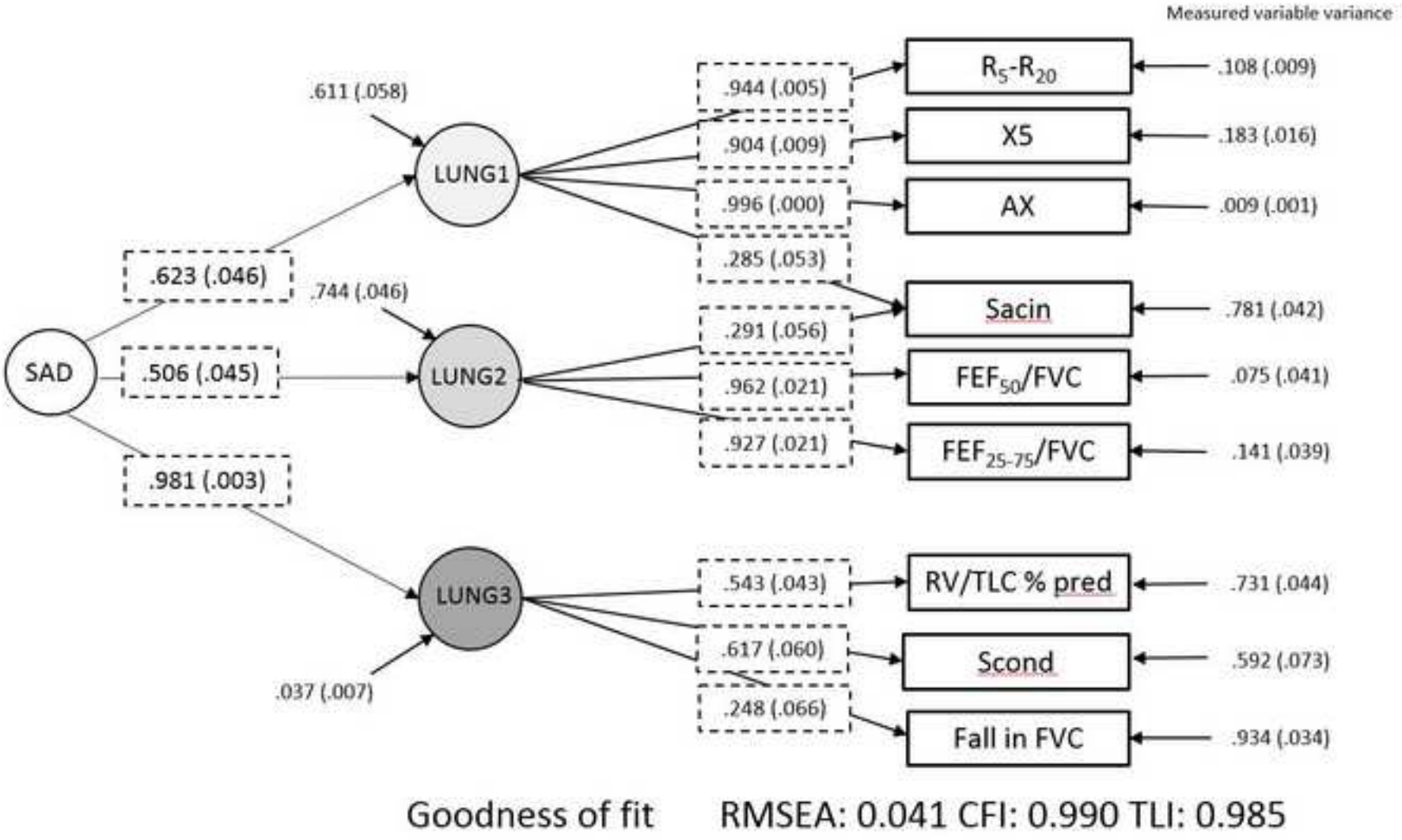

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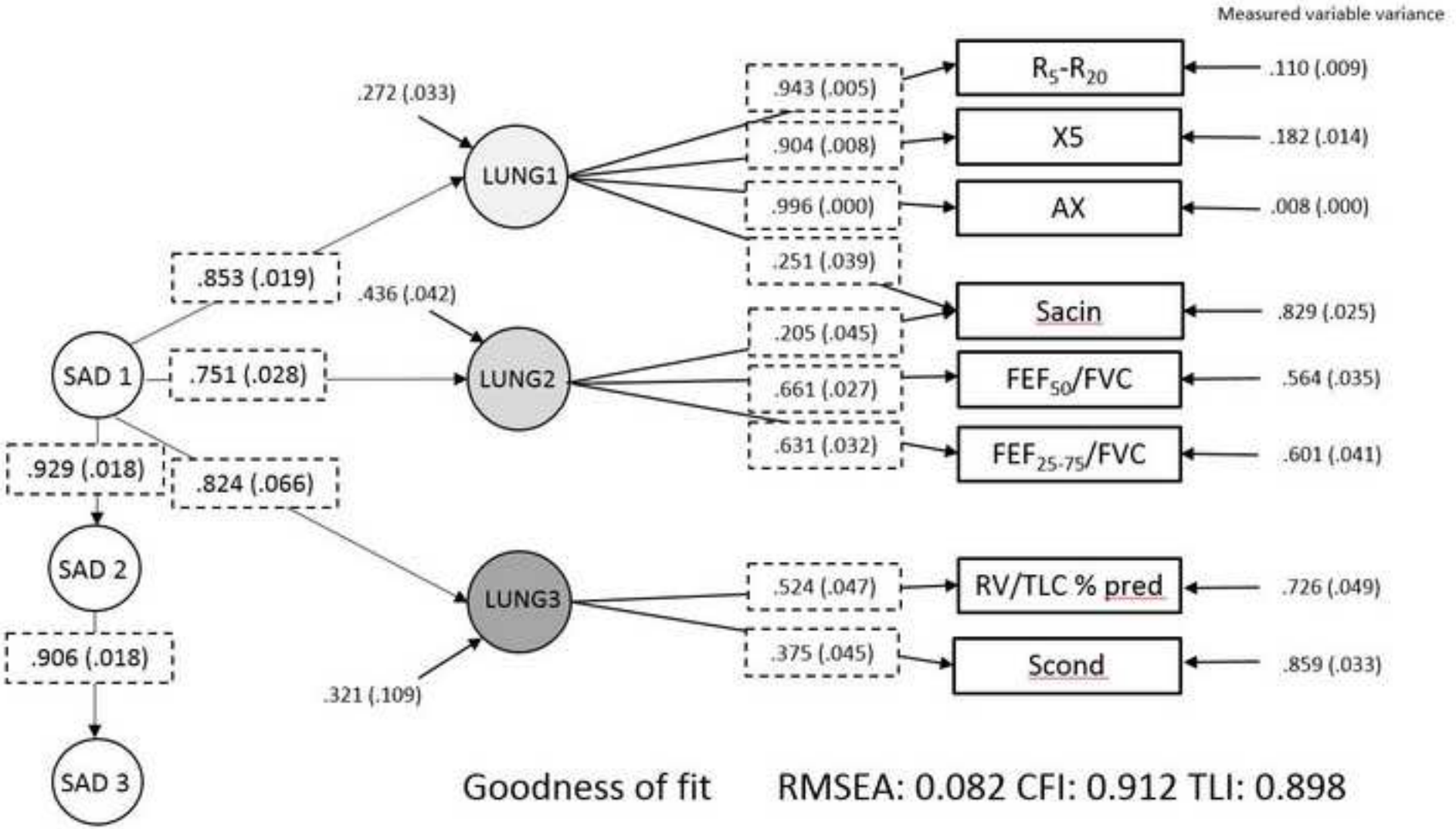

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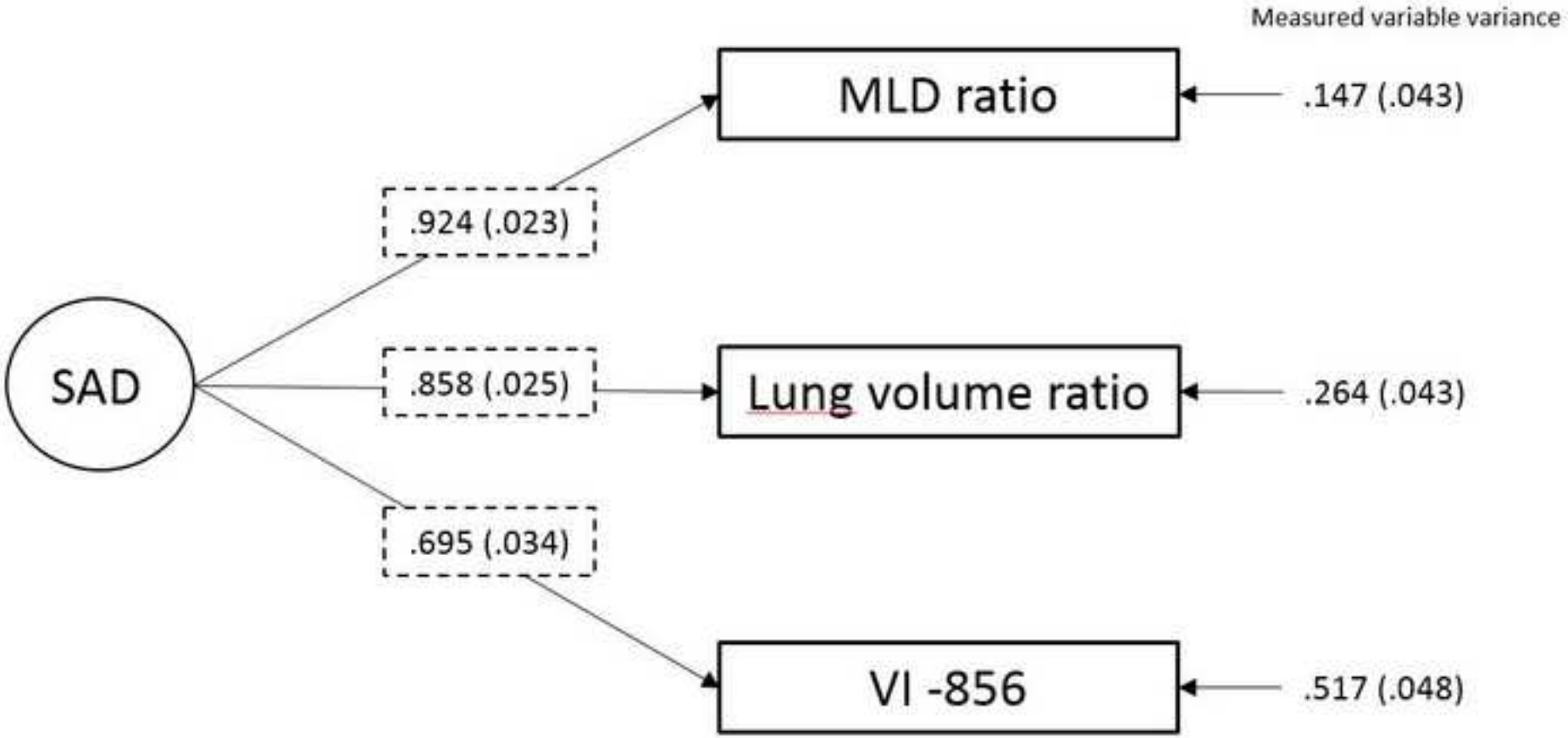

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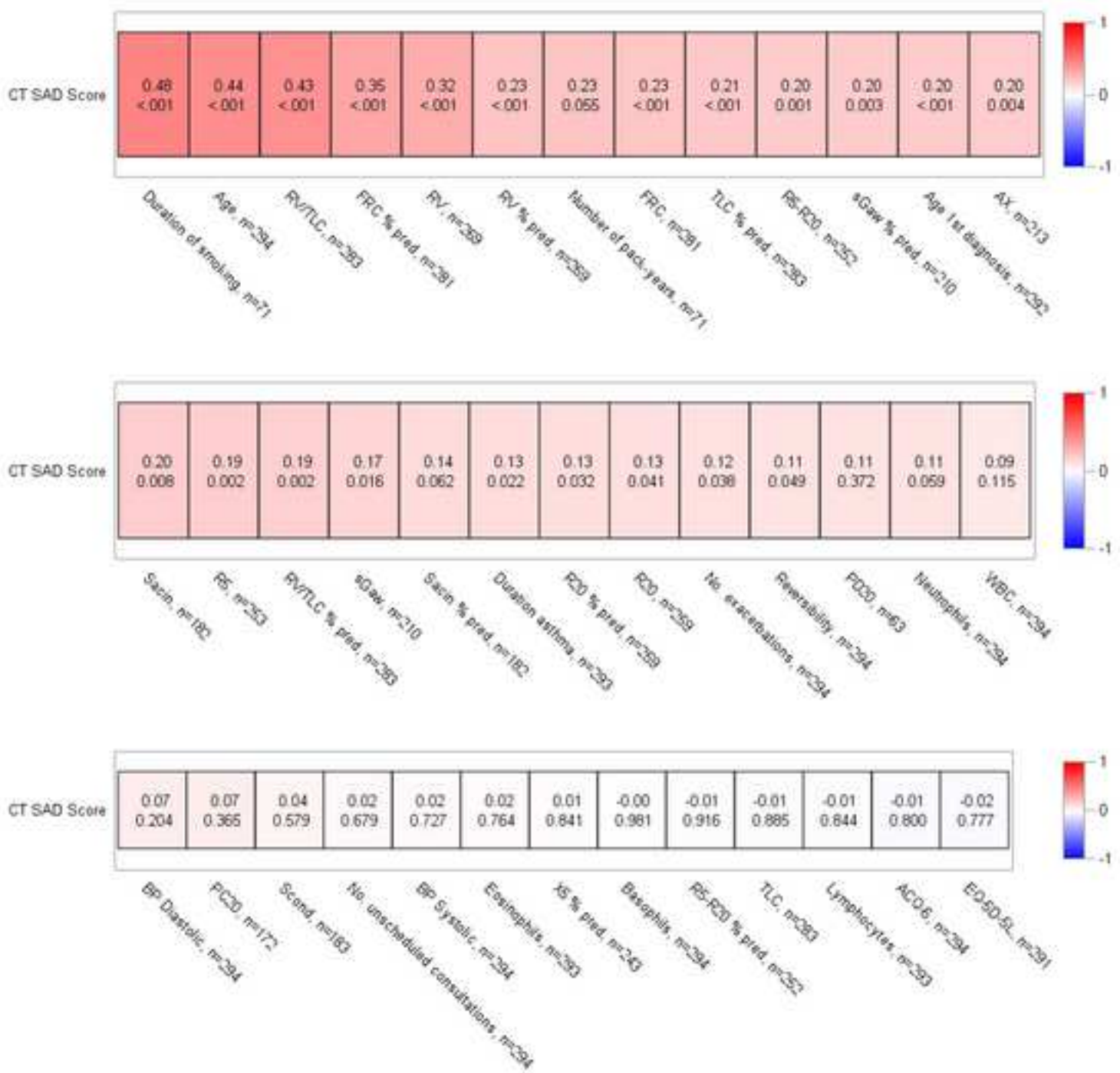

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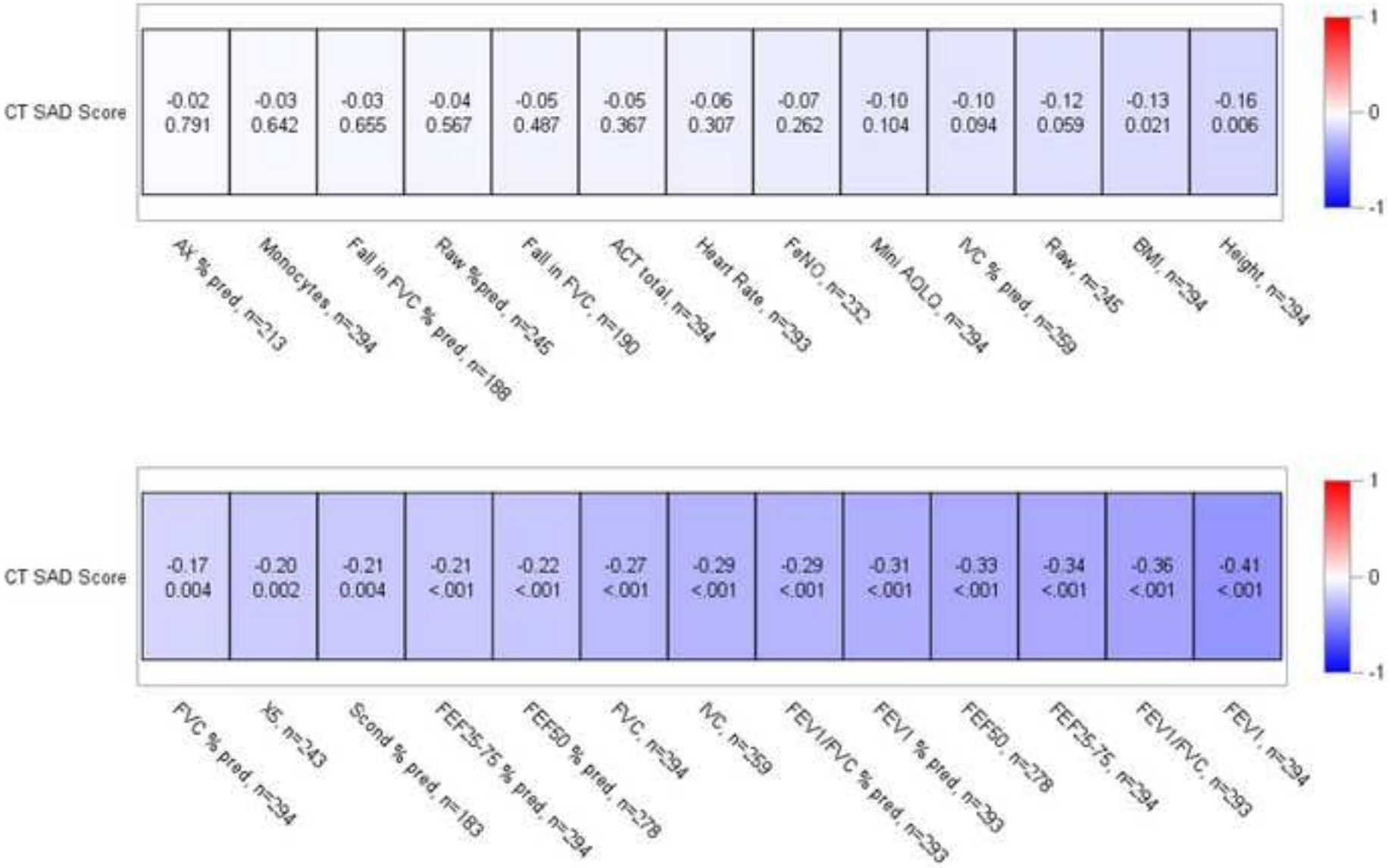

PubMed collaborator list

name	surname
Emilio	Pizzichini
Alberto	Cukier
Rafael	Stelmach
Ronald	Olivenstein
Qingling	Zhang
Philipp	Badorrek
Christian	Gessner
Nicola	Scichilone
Alfredo	Chetta
Pierluigi	Paggiaro
Stefano	Milleri
Mariella	D'Amato
Antonio	Spanevello
Maria Pia	Foschino
Willem Germen	Boersma
Marielle	Broeders
J.Sebastiaan	Vroegop
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